Attorney Docket 21715/1010

COMPOSITIONS AND METHODS INVOLVING AN ESSENTIAL STAPHYLOCOCCUS AUREUS GENE AND ITS ENCODED PROTEIN

RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Patent Application No. 09/470,512, filed December 22, 1999, which claims the benefit of U.S. Patent Application No. 09/407,804, filed September 28, 1999, and U.S. Provisional Application No. 60/110,992, filed December 3, 1998.

ABSTRACT OF THE DISCLOSURE

This invention relates to newly identified polynucleotides and polypeptides, and their production and uses, as well as their variants, agonists and antagonists, and their uses. In particular, the invention relates to polynucleotides and polypeptides of a *Staphylococcus aureus* (*S. aureus*) DnaI related protein, as well as its variants, hereinafter referred to as "*S. aureus* DnaI", "*S. aureus* DnaI polypeptide(s)", and "*S. aureus* dnaI polynucleotides" as the case may be. Also, the invention relates to a specific interaction between the *S. aureus* DnaI related protein or specific regions thereof, and a growth-inhibitory protein encoded by the *S. aureus* bacteriophage 77 genome. The phage open reading frame (ORF) product interacts with amino acids 150 – 313 of *S. aureus* DnaI polypeptide, and the invention relates to the use of this interaction target site as the basis of drug screening assays. In addition, the invention relates to polynucleotides and polypeptides of a protein complex containing *S. aureus* DnaI and DnaC related proteins, as well as their variants.

FIELD OF THE INVENTION

The invention relates to bacterial and bacteriophage genes.

BACKGROUND OF THE INVENTION

The Staphylococci make up a medically important genera of microbes known to cause several types of diseases in humans. *S. aureus* is a Gram positive organism which can be found on the skin of healthy human hosts. It is responsible for a large number of bacteremias, where its portal of entry can be the skin, lungs, urinary tract or infected intravascular devices (Steinberg *et al.*, (1996)) Clin. Infect. Dis. **23:** 255-259; Røder *et al.*, (1999) Arch. Intern. Med. **159:** 462-469). It can cause fatal endocarditis or damage to the heart and, due to its exotoxin, can cause death via "Toxic Shock" (Frimodt-Møller *et al.*, (1997) Clin. Microbiol. Infect. **3:** 297-305; Sanabria *et al.*, (1990) Arch. Intern. Med. **150:** 1305-1309).

Only *S. aureus* and *Staphylococcus epidermidis*, of the nineteen species of *Staphylococcus* described in Bergey's Manual (1992), have significant interactions with humans. They are among the normal flora of humans, and are found on nasal passages, skin and mucous membranes. *S. aureus*, when pathogenic in humans, can cause a number of suppurative (pus-forming) infections, as well as food poisoning, endocarditis, and toxic shock syndrome.

S. aureus causes superficial skin lesions, such as boils, styes and furunculosis; more serious infections include pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections, in addition to osteomyelitis and endocarditis. *S. aureus* is also a major cause of hospital

acquired (nosocomial) infection of surgical wounds and infections associated with inserted and implanted medical devices. Lastly, *S. aureus* causes food poisoning through the release of enterotoxins into food, and toxic shock syndrome through the release of superantigens into the blood stream. *S. aureus* also secretes two types of toxin with superantigen activity: **1)** enterotoxins, of which there are six antigenic types (named SE-A, B, C, D, E and G) and **2)** toxic shock syndrome toxin (TSST-1).

S. aureus has been successfully treated with the penicillin derivative Methicillin in the past, but is now becoming increasingly resistant (MRSA - Methicillin Resistant *S. aureus*) to this antibiotic (Harbath *et al.*, (1998) Arch. Intern. Med. **158:** 182-189.). For example, *S. aureus* endocarditis mortality can range from 26-45%, and combined ß-lactam/aminoglycoside therapy is proving increasingly ineffective in disease eradication (Røder *et al.*, (1999) Arch. Intern. Med. **159:** 462-469). However, MRSA infections continue to be sensitive to treatment with vancomycin, which is the drug of last resort. Infections caused by MRSA have been increasing in children and adults; isolates have been found in 97% of all large, university-based teaching hospitals in the United States. Since 1996, three cases of vancomycin resistant *S. aureus* have been reported. This new strain represents a particularly dangerous development of an aggressive bacterial pathogen which does not respond to any known antibiotic. The emergence of resistance to vancomycin has the potential to result in untreatable (and thus fatal) *S. aureus* infections.

It is no longer uncommon to isolate *S. aureus* strains which are resistant to most of the standard antibiotics, and thus there is an unmet medical need and demand for new anti-microbial agents, vaccines, drug screening methods, and diagnostic tests for this organism.

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SUMMARY OF THE INVENTION

The present invention relates to DnaI and DnaI-related proteins, in particular *S. aureus* DnaI polypeptides and dnaI polynucleotides, recombinant materials and methods for their production. The invention also relates to a pair of interacting proteins, a growth-inhibitory (or inhibitor) bacteriophage 77 ORF 104 gene product that interacts with the *S. aureus* DnaI polypeptide, the interacting regions of the *S. aureus* DnaI related protein and the protein encoded by the *S. aureus* bacteriophage 77 ORF 104, forming the basis for screening assays. It also relates to polynucleotides and polypeptides of a multiprotein complex believed to be involved in initiation of DNA replication containing DnaI as a subunit, and also may include DnaC and related proteins, as well as variants of them. In another aspect, the invention relates to methods for using such polypeptides and polynucleotides, including treatment of microbial diseases, amongst others. In a further aspect, the invention relates to methods for identifying agonists and antagonists using the materials provided by the invention, and for treating microbial infections and conditions associated with such infections with the identified agonist or antagonist compounds. In a still further aspect, the invention relates to diagnostic assays for detecting diseases associated with microbial infections and conditions associated with such infections, such as assays for detecting DnaI expression or activity.

The invention encompasses a method of identifying a compound that is active on a polypeptide comprising the amino acid sequence of SEQ ID NO: 16. The method comprises contacting a candidate compound with the polypeptide, and detecting binding of the candidate

compound to the polypeptide, wherein detection of binding is indicative that the compound is active on the polypeptide.

In one embodiment, the step of detecting comprises the step of measuring the binding of a candidate compound, wherein the compound is directly or indirectly detectably labeled, to the polypeptide.

In another embodiment, the step of detecting comprises measurement by phage display.

In another embodiment, the step of detecting comprises measurement by surface plasmon resonance.

In another embodiment, the step of detecting comprises measurement by FRET.

In another embodiment, the step of detecting comprises measurement of fluorescence polarization changes.

In another embodiment, the step of detecting comprises a scintillation proximity assay.

In another embodiment, the step of detecting comprises a biosensor assay.

In another embodiment, the active compound is selected from the group consisting of a small molecule, a peptidomimetic compound, and a fragment or derivative of a bacteriophage inhibitor protein.

In another embodiment, the active compound is a peptide synthesized by a recombinant expression system and purified, or artificially synthesized.

The invention also encompasses a method of identifying a compound that is active on a polypeptide comprising the amino acid sequence of SEQ ID NO: 16, the method comprising the steps of contacting a first and a second polypeptide in the presence and absence of a candidate

compound, wherein the first polypeptide comprises the amino acid sequence of SEQ ID NO: 16 or a fragment or variant thereof that specifically binds phage 77ORF104 and the second polypeptide comprises phage 77 ORF 104 or a domain thereof that specifically binds a polypeptide of SEQ ID NO: 16, and detecting the binding of the first and second polypeptides to each other, wherein a decrease in the binding of the first and the second polypeptides in the presence of the candidate compound relative to the binding in the absence of the candidate compound identifies the candidate compound as a compound that is active on a polypeptide comprising the amino acid sequence of SEQ ID NO: 16.

In one embodiment, the first or the second polypeptide is directly or indirectly detectably labeled.

In another embodiment, the step of detecting comprises measurement by phage display.

In another embodiment, the step of detecting comprises measurement by surface plasmon resonance.

In another embodiment, the step of detecting comprises measurement by FRET.

In another embodiment, the step of detecting comprises measurement of fluorescence polarization changes.

In another embodiment, the step of detecting comprises a scintillation proximity assay.

In another embodiment, the step of detecting comprises a biosensor assay.

The invention further encompasses an agonist or an antagonist of the activity of a DnaI polypeptide or a gene encoding the polypeptide.

The invention further encompasses a method of identifying a compound that is active on a DnaI polypeptide, comprising the steps of contacting a candidate compound with cells expressing a polypeptide comprising SEQ ID NO: 16 and detecting DnaI activity in the cells, wherein a decrease in activity relative to DnaI activity in cells not contacted with a candidate compound is indicative of inhibition of DnaI activity.

The invention further encompasses a method of making an antibacterial compound, comprising the steps of: a) determining whether a candidate compound is active on a polypeptide comprising the amino acid sequence of SEQ ID NO: 16 or a gene encoding the polypeptide; and b) synthesizing or purifying the candidate compound in an amount sufficient to provide a therapeutic effect when administered to an organism infected by a bacterium naturally producing a polypeptide comprising the amino acid sequence of SEQ ID NO: 16.

In one embodiment, the candidate compound is selected from the group consisting of a small molecule, a peptidomimetic compound, and a fragment or derivative of a bacteriophage inhibitor protein.

In one embodiment, the candidate compound is a peptide synthesized by a recombinant expression system and purified, or artificially synthesized.

The invention further encompasses a method for inhibiting a bacterium, comprising contacting the bacterium with a compound active on a polypeptide comprising the amino acid sequence of SEQ ID NO: 16 or a gene encoding the polypeptide.

In one embodiment, the step of contacting is performed in vitro.

In another embodiment, the step of contacting is performed in vivo in an animal.

In another embodiment, the compound is selected from the group consisting of a small molecule, a peptidomimetic compound, and a fragment or derivative of a bacteriophage inhibitor protein.

In another embodiment, the compound is a peptide synthesized by a recombinant expression system and purified, or is artificially synthesized.

The invention further encompasses a method for treating a bacterial infection in an animal suffering from an infection, comprising administering to the animal a therapeutically effective amount of a compound active on a polypeptide comprising the amino acid sequence of SEQ ID NO: 16 or a gene encoding the polypeptide. The animal is preferably, but not necessarily a mammal, more preferably a human.

In one embodiment, the compound is selected from the group consisting of a small molecule, a peptidomimetic compound, and a bacteriophage inhibitor protein.

The invention further encompasses a method of prophylactic treatment to prevent bacterial infection comprising contacting an indwelling device with a compound active on a polypeptide comprising the amino acid sequence of SEQ ID NO: 16 before its implantation into a mammal, such contacting being sufficient to prevent *S. aureus* infection at the site of implantation.

The invention further encompasses a method of prophylactic treatment to prevent infection of an animal by a bacterium comprising administering to the animal a compound that is active on a polypeptide comprising the amino acid sequence of SEQ ID NO: 16 or a gene

encoding the polypeptide in an amount sufficient to reduce adhesion of the bacterium to a tissue surface of the mammal.

The invention further encompasses a method of diagnosing in an individual an infection with *Staphylococcus aureus*, comprising: determining the presence in the individual of a polypeptide comprising the amino acid sequence of SEQ ID NO: 16.

In one embodiment, the determining step comprises contacting a biological sample of the individual with an antibody specific for an epitope present on a polypeptide comprising the amino acid sequence of SEQ ID NO: 16.

The invention further encompasses a method of diagnosing in an individual an infection with Staphylococcus aureus, comprising determining the presence in the individual of a nucleic acid sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 16.

In one embodiment, the determining step comprises contacting a nucleic acid sample of said individual with an isolated, purified or enriched nucleic acid probe of at least 15 nucleotides in length that hybridizes under stringent hybridization conditions with the sequence of SEQ ID NO: 1, or the complement of such probe.

The invention further encompasses an isolated, purified or enriched polynucleotide comprising a nucleotide sequence that has at least 55% identity to the sequence of SEQ ID NO: 1, or the complement of said nucleotide sequence.

The invention further encompasses an isolated, purified or enriched polynucleotide consisting of nucleotides 448-942 of SEQ ID NO: 1, herein referred to as SEQ ID NO: 17,

comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO: 16 or the complement of such nucleotide sequence.

The invention further encompasses an isolated, purified or enriched polynucleotide consisting of the sequence of SEQ ID NO: 17.

The invention further encompasses an isolated, purified or enriched polypeptide having at least 55% identity to the amino acid sequence of SEQ ID NO: 16.

The invention further encompasses an isolated, purified or enriched polypeptide of at least 50 amino acids in length having at least 50 % identity to the amino acid sequence of SEQ ID NO: 16.

The invention further encompasses an isolated, purified or enriched polypeptide having at least 70% similarity to the amino acid sequence of SEQ ID NO: 16.

The invention further encompasses an isolated, purified or enriched polypeptide of at least 20 amino acids in length having at least 60% similarity to the amino acid sequence of SEQ ID NO: 16.

The invention further encompasses an isolated polypeptide comprising the amino acid sequence of SEQ ID NO: 16.

The invention further encompasses an isolated polypeptide consisting of the amino acid sequence of SEQ ID NO: 16.

The invention further encompasses an isolated, purified or enriched antibody specific for a polypeptide comprising SEQ ID NO: 16.

The invention further encompasses a composition comprising two polypeptides, a bacteriophage 77 ORF 104 polypeptide and a polypeptide comprising the amino acid sequence of SEQ ID NO: 16 or a variant thereof that specifically binds phage 77 ORF 104 polypeptide.

The invention further encompasses a composition comprising a nucleic acid encoding bacteriophage 77 ORF 104 and a nucleic acid comprising SEQ ID NO: 17 or a variant thereof that encodes a polypeptide that specifically binds bacteriophage 77 ORF 104 polypeptide.

Further features and advantages of the invention will become more fully apparent in the following description of the embodiments and drawings thereof, and from the claims.

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BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the nucleotide (A; SEQ ID NO: 1) and amino acid (B; SEQ ID NO: 2) sequences of *S. aureus* DnaI.

Fig. 2 shows the complete nucleotide sequence of the *S. aureus* bacteriophage 77 genome (SEQ ID NO: 3).

Fig. 3 shows an ORF map of the *S. aureus* bacteriophage 77 genome.

Fig. 4 shows the nucleotide (A; SEQ ID NO: 4) and the amino acid (B; SEQ ID NO: 5) sequences of *S. aureus* bacteriophage 77 ORF 104.

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Fig. 5 shows the predicted tryptic peptide masses of the ORF identified in the University of Oklahoma *S aureus* genomic database that closely matches the tryptic peptide profile of the polypeptide bound by 77ORF104.

Fig. 6 shows alignments of *B. subtilis* DnaC sequences with the homologous sequences from *S. aureus*. A) shows an alignment of *B. subtilis* dnaC polynucleotide sequence (SEQ ID NO: 6) with the homologous *S. aureus* dnaC polynucleotide sequence (SEQ ID NO: 7) identified by BLAST searching the *S. aureus* database at http://www.tigr.org with the *B. subtilis* dnaC sequence. B) shows an alignment of *B. subtilis* DnaC amino acid sequence (SEQ. ID NO: 8) with the predicted amino acid sequence of the polypeptide (SEQ ID NO: 9) encoded by the *S. aureus* dnaC polynucleotide sequence shown in Fig. 6A.

Fig. 7 shows the killing potential of bacteriophage 77 ORF 104 and the expression vector used to induce its expression in *S. aureus*: A) Schematic diagram of expression vector pT/ORF used to induce expression of 77ORF104 in *S. aureus* cells; B) Results of a screen to assess the killing potential of 77ORF104 when expressed in *S. aureus* grown on semi-solid support media; and C) Results showing the inhibitory potential of 77ORF104 when expressed in *S. aureus* in liquid media.

Fig. 8 shows affinity chromatography using GST and GST/ORF104 as ligands with the *S. aureus* extract prepared by French pressure cell lysis and sonication. Eluates from affinity columns containing the GST and GST/ORF104 ligands at 0, 0.1, 0.5, 1.0, and 2.0 mg/ml resin were resolved by 12.5% SDS-PAGE. Proteins were visualized by silver staining. Micro-columns were eluted with: A) ACB containing 1 M NaCl; B) 250 mM NaCl; C) 1% Triton X-100; and D) 1% SDS. Each molecular weight marker (Mr) is approximately 100 ng. The lanes labeled ACB indicate eluates from a 2.0 mg/ml ligand column loaded only with ACB buffer containing 75 mM NaCl. The arrows indicate bands specifically interacting with GST/ORF104.

Fig. 9 shows affinity chromatography with GST and GST/ORF104 as ligands with the *S. aureus* extract prepared by lysis with lysostaphin digestion and sonication. Eluates from affinity columns containing the GST and GST ORF104 ligands at 0, 0.1, 0.5, 1.0, and 2.0 mg/ml resin were resolved by 12.5% SDS-PAGE. Micro-columns were sequentially eluted with 75 mM ACB containing 1% Triton X-100, 250 mM NaCl, 1 M NaCl ACB, and 1% SDS. The elution profile obtained with 1% SDS is shown. Each molecular weight marker (Mr) is approximately 100 ng. The lanes labeled ACB indicate eluates from a 2.0 mg/ml ligand column loaded only with ACB buffer containing 75 mM NaCl. Lanes labeled C and L are corresponding elutions from columns containing GST and GST/ORF104 at 2.0 mg/ml from Figure 8. The arrow indicates a polypeptide specifically interacting with GST/ORF104.

Fig. 10 shows affinity chromatography with ORF104 (GST removed) as ligand with the *S. aureus* extract prepared by lysis with lysostaphin digestion and sonication (Lys extract) and French pressure cell and sonication (FP/S extract). Eluates from affinity columns containing the ORF104 ligand at 0, 0.1, 0.5, 1.0, and 2.0 mg/ml resin were resolved by 12.5% SDS-PAGE and the gel was stained with silver nitrate. Micro-columns were sequentially eluted with: ACB containing 1% Triton X-100; 250 mM NaCl; 1M NaCl; and 1% SDS. The elution profile obtained with 1% SDS is shown. Each molecular weight marker (Mr) is approximately 100 ng. The lanes labeled ACB indicate eluates from a 2.0 mg/ml ligand column loaded only with ACB buffer containing 75 mM NaCl. The arrow indicates a polypeptide specifically interacting with GST/ORF104.

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Fig. 11 shows results of a tryptic peptide mass spectrum analysis showing relatedness between the interacting protein eluted with Triton X-100 (indicated by arrow in Fig. 8C) and the interacting protein eluted with 1% SDS (indicated by arrow in Fig. 8D). Of note are the tryptic peptides having monoisotopic MH+ masses of 1351.8, 1412.7, and 1617.8 Da.

Fig. 12 shows the results of yeast two hybrid analyses designed to test the interaction of *S. aureus* DnaI and 77 ORF 104. A) Construction of the yeast pGADT7 vector expressing the polypeptide Gal4 activation domain (GAD) fused to the *S. aureus* DnaI. B) Construction of the yeast pGBKT7 vector expressing the polypeptide Gal4 DNA binding domain (GBK) fused to phage 77 ORF104. 77 ORF 104 and DnaI were also cloned into pGADT7 and pGBKT7, respectively (not shown). C) Yeast two-hybrid assay. D) Yeasts were co-transformed, as indicated (No 1 to 6), in the presence or in the absence of control vectors. pGADT7-T and pGBKT7-53 (NO 1) are positive control for protein:protein interaction and pCL1 (NO 4) is an active Gal4 transcription factor. Co-transformants were plated in parallel on yeast synthetic medium (SD) supplemented with amino acid drop-out lacking tryptophan and leucine (TL minus) and on SD supplemented with amino acid drop-out lacking tryptophan, histidine, adenine and leucine (THAL minus). Co-transformants harboring 77 ORF104 polypeptide only grew on selective THAL minus media in the presence of DnaI (NO 5 and 6). E) Results of the luminescent β-galactosidase enzymatic assays with protein extracts from the same co-transformants (NO 1 to 6).

Fig. 13 shows inhibition of S. aureus DNA synthesis by bacteriophage 77 ORF 104 protein.

Fig 14 shows the interaction between partial proteolysis fragments of DnaI and ORF 104 from *S. aureus* bacteriophage 77. Partial proteolytic fragments generated by A) endoproteinase GluC or B) chymotrypsin were subjected to affinity chromatography using columns containing either 0 or 2.0 mg/ml of 77ORF104 protein. Partial proteolytic fragments interacting with the 77ORF104 and not the control column were excised for peptide mapping. Lanes are indicated as Mr, molecular weight markers; L, load; FT, flowthrough; 1, 1 M NaCl elution; 2, 1% SDS elution; ACB, affinity chromatography buffer. The interacting bands excised for peptide mapping are indicated according to the apparent Mr by SDS-PAGE, bands not interacting are indicated with (-). C) List of identified DnaI proteolytic fragments interacting with 77 ORF 104. Partial proteolytic fragments interacting with 77ORF104 were purified by reverse phase, analyzed with MALDI-TOF, and the observed high molecular weight fragments mapped to the corresponding amino acid sequence of SEQ ID NO: 2. The minimal domain of DnaI interacting with 77 ORF 104 as determined by partial proteolysis with chymotrypsin is amino acids 131 to 313 and with endoproteinase Glu-C is amino acids 119 to 313 of SEQ ID NO: 2.

Fig. 15 shows the amino acid sequence of the DnaI fragments tested in yeast two-hybrid system for interaction with 77ORF104. SEQ ID NO: 16 contains the amino acids 150 to 313 of SEQ ID NO: 2 and SEQ ID NO: 17 contains the corresponding nucleotides 448 to 942 of SEQ ID NO: 1. SEQ ID NO: 18 contains the amino acids 64 to 313 of SEQ ID NO: 2.

Fig. 16 shows the results of the yeast two-hybrid analysis that were designed to test the interaction between fragments of DnaI and 77 ORF 104. Fragments of *S. aureus* DnaI were cloned into pGADT7 vector. Yeasts were co-transformed with the plasmids indicated from No 1

to 6. pGBKLam and 77pGADORF13 are control vectors driving the expression of non-interacting proteins. Co-transformants were plated in parallel on THAL minus SD medium and on TL minus SD medium. Co-transformants bearing 77ORF104 only grew on selective THAL minus media in the presence of DnaI or DnaI fragments (No 1, 3 and 5). D) Representation of fragments of DnaI interacting with 77ORF104. The minimal domain of DnaI interacting with 77 ORF 104 as determined by yeast two-hybrid analysis is amino acids 150 to 313.

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DESCRIPTION OF THE INVENTION

The invention is based on the discovery of an essential gene and its encoded polypeptide in *S. aureus* and portions thereof useful in screening, diagnostics, and therapeutics. The invention also relates to *S. aureus* DnaI polypeptides and polynucleotides as described in greater detail below, and to a pair of polynucleotides encoding a pair of interacting polypeptides, and the pair of polypeptides themselves, or interacting domains thereof, where the pair includes a *S. aureus* DnaI polypeptide and a 77 ORF 104 polypeptide. Also, the invention relates to polynucleotides and polypeptides of a protein complex, thought to be involved in initiation of DNA replication, containing DnaI and DnaC related proteins, as well as their variants. In particular, the invention relates to polypeptides and polynucleotides of a DnaI of *S. aureus*, which is related by amino acid sequence homology to *B. subtilis* DnaI polypeptide. The invention relates especially to DnaI having the nucleotide and amino acid sequences disclosed as SEQ ID NO: 1 and SEQ ID NO: 2, respectively. The sequences presented as SEQ ID NOs: 1 and 2 represent an exemplification of the invention, since those of ordinary skill will recognize that

such sequences can be usefully employed in polynucleotides in general, including ribopolynucleotides.

We have used the methodology of two previous inventions (U.S. Patent Application Serial No. 09/407.804, filed Sept. 28, 1999, and U.S. Provisional Patent Application 60/110,992 filed December 3, 1998) to identify and characterize an essential polynucleotide and polypeptide sequence from *S. aureus*. Thus, the present invention provides polynucleotide and polypeptide sequences isolated from *S. aureus* that can be used in a drug screening assay to identify compounds with anti-microbial activity. The polynucleotide and polypeptide sequences can be isolated using a method similar to those described herein, or using another method. In addition, such polynucleotide and polypeptide sequences can be chemically synthesized.

Definitions

The phrase "active on", with reference to a particular cellular target, such as the product of a particular gene, means that the target is an important part of a cellular pathway which includes that target and that an agent or compound acts on that pathway. Thus, in some cases the agent or compound may act on a component upstream or downstream of the stated target, including a regulator of that pathway or a component of that pathway. In general, an antibacterial agent is active on an essential cellular function, often on a product of an essential gene.

As used herein, the terms "inhibit", "inhibition", 'inhibitory", and "inhibitor" all refer to a function of reducing a biological activity or function. Such reduction in activity or function can, for example, be in connection with a cellular component (e.g., an enzyme), or in connection with

a cellular process (e.g., synthesis of a particular protein), or in connection with an overall process of a cell (e.g., cell growth). In reference to cell growth, the inhibitory effects may be bacteriocidal (killing of bacterial cells) or bacteriostatic (i.e. - stopping or at least slowing bacterial cell growth). The latter slows or prevents cell growth such that fewer cells of the strain are produced relative to uninhibited cells over a given time period. From a molecular standpoint, such inhibition may equate with a reduction in the level of, or elimination of, the transcription and/or translation of a specific bacterial target(s), or reduction or elimination of activity of a particular target biomolecule.

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As used herein, the term "DnaI polypeptide" refers to a polypeptide encompassing *S. aureus* DnaI (SEQ ID NO: 2) or an active domain of *S. aureus* DnaI. As used herein, the term "active domain of *S. aureus* DnaI" is a polypeptide fragment or portion of *S. aureus* DnaI that retains an activity of *S. aureus* DnaI. The term "DnaI polypeptide" is meant to encompass *S. aureus* DnaI or an active domain of *S. aureus* DnaI that is fused to another, non-DnaI polypeptide sequence.

"DnaI activity" is defined as one or more of the following:

A) The activity of a polypeptide having the *S. aureus* DnaI sequence provided herein, a fragment or analog thereof or a protein comprising a *S. aureus* DnaI polypeptide that directly interacts with bacteriophage 77 ORF 104 protein or a DnaI-binding fragment thereof in a manner that results in at least a 10-fold reduction of ^{3}H-thymidine incorporation in a bacterial DNA replication assay relative to ^{3}H-thymidine incorporation in an assay lacking bacteriophage 77 ORF 104 or a DnaI-binding fragment thereof.

To assay for DnaI activity by ^{3}H-thymidine incorporation, the level of radiolabeled thymidine incorporation into DNA is measured in *S. aureus* cells expressing an arsenite-inducible 77 ORF104 construct in the presence or absence of 5 μM sodium arsenite. Samples (0.5 ml) are withdrawn from cultures at appropriate time intervals and mixed with 4.5 μl of labeling solution (0.2 μCi/ml of ^{3}H-thymidine (73 Ci/mmol, NEN Life Science Products, Inc.) and 70 pmol of unlabeled thymidine). After 15 minutes of reaction, incorporation is stopped by adding 5 μl of 0.2% NaN_3 and 5 μl of 30 μg/ml unlabeled thymidine. Samples are precipitated with 10% (w/v) trichloroacetic acid and filtered through glass fiber filters (GF-C, Whatman). The results are expressed as ^{3}H -thymidine counts incorporated, normalized to the OD of the culture.

B) The activity of a polypeptide having the *S. aureus* DNA sequence provided herein, or a fragment or analog thereof, or a protein comprising a *S. aureus* DnaI polypeptide that is necessary for at least a 10% inhibition of plasmid replication by bacteriophage 77 ORF 104 protein in the plasmid DNA replication assay. This assay is as follows, the plasmid pC194 replicates in *S. aureus* by a rolling circle mechanism. The single-stranded origin, *sso*, of pC194 is involved in the synthesis of the lagging strand of DNA. The plasmid pADG6406 is a derivative of pC194 lacking *sso*. The absence of *sso* leads to the accumulation of single-stranded plasmid DNA. The single stranded initiation site, *ssiA*, is located on the lagging strand of pAM 1, and is a site for replicative primosome assembly. *SsiA* was inserted into plasmid pADG6406. *S. aureus* cells carrying plasmids are grown to mid-log phase and their total DNA is extracted and analyzed by Southern hybridization using ^{32}P-labeled plasmid DNA as probe. The presence

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of pADG6406 with *ssiA* is associated with a decrease in the ratio of single-stranded to double-stranded plasmid DNA compared to the ratio in cells bearing the same plasmid lacking the *ssiA* insert. This system is used to measure the effect of 77 ORF 104 expression on single-stranded DNA synthesis. A plasmid containing 77 ORF 104 under an arsenite-inducible promoter is transformed into *S. aureus* harboring pADG6406. The ratio of single-stranded to double-stranded DNA of pADG6406 is measured in the presence and absence of sodium arsenite. An increase in the ratio of single-stranded to double-stranded DNA of 10% or more in the presence of 77 ORF 104 indicates an effect on DnaI activity.

C) The activity of a polypeptide having the *S. aureus* sequence provided herein, a fragment or analog thereof, or a protein comprising a *S. aureus* DnaI polypeptide in the loading of *S. aureus* DnaC helicase onto replicative primosomes. The following helicase assay can be adapted from an in vitro assay with SPP1 phage G38P (DnaA), G39P (DnaI) and G40P (DnaC) polypeptides (Ayora et al., 1999, J. Mol. Biol. 288: 71-85) Helicases are capable of unwinding DNA with a 5' to 3' unwinding polarity. To determine the role of *S. aureus* DnaI on the helicase unwinding activity, an annealed substrate with a 3' single-stranded (ss) DNA tail (preformed fork) is incubated with a constant quantity of purified DnaC helicase and increasing amounts of either purified DnaI, DnaA or preformed DnaA-DnaI complex. The reaction mixture is subjected to conditions that support helicase activity. The reaction contains 50 mM NaCl, 1 mM ATP, 50 μg/ml BSA and 0.24 nM ^{32}P-labeled oligomer annealed to M13 ssDNA offered as substrate. The DNA molecule in the reaction mixture is analyzed for whether it is converted to single-stranded (ss) DNA. The reaction is stopped by the addition of 5 μl of stopping solution (100 mM EDTA,

2% (w/v) SDS in DNA loading buffer (Sambrook 1989)) and subsequently loaded onto a 10% non-denaturing PAGE gel. The gel is run and dried prior to autoradiography. The ratio of the oligo released from the M13 ssDNA is evaluated.

D) The binding or interaction of a polypeptide comprising the amino acid sequence of SEQ ID NO: 16, provided herein, to bacteriophage 77 ORF 104 protein or a portion thereof capable of binding a polypeptide comprising the amino acid sequence of SEQ ID NO. 16. The interaction or binding of a polypeptide comprising the amino acid sequence of SEQ ID NO. 16 and a binding portion of bacteriophage 77 ORF 104 may be between isolated polypeptides consisting essentially of the sequence necessary for binding, or, alternatively, the respective polypeptide sequence may be comprised within a larger polypeptide. A number of methods, useful in the invention, to measure the binding of bacteriophage 77 ORF 104 to a polypeptide comprising the amino acid sequence of SEQ ID NO: 16 are described below. For example, Phage display is a powerful quantitative assay to measure protein:protein interaction using colorimetric ELISA (enzyme-linked immunosorbent assay). Surface plasmon resonance assays can be used as a quantitative method to measure binding between two molecules by the change in mass near an immobilized sensor caused by the binding of one protein from the aqueous phase to a second immobilized on the sensor. An additional useful binding assay is Fluorescence Resonance Energy Transfer (FRET), in which the close proximity of two fluorophores, each bound to a separate molecule, causes the excitation spectrum of one fluorophore to overlap with the excitation spectrum of the second, and thus dual fluorescence following excitation of only one fluorophore is indicative of binding. An additional assay useful in the present invention is

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fluorescence polarization, in which the quantifiable polarization value for a given fluorescently-tagged molecule is altered upon binding to a second molecule. A scintillation proximity assay can also be used to measure binding of a polypeptide comprising the amino acid sequence of SEQ ID NO: 16 and bacteriophage 77ORF104, in which the emmitance of radioactive particles is altered upon binding. Additionally, binding can be evaluated by a Bio Sensor assay, which is based on the ability of the sensor to register changes in admittance induced by ion-channel modulation following binding. A further assay which can be used to measure the binding of a polypeptide comprising the amino acid sequence of SEQ ID NO: 16 and bacteriophage 77 ORF 104 is the yeast two hybrid assay, in which the binding of the two polypeptides within the context of two fusion proteins expressed in yeast cells, permits the expression of reporter molecules which, in turn produces a measurable, or detectable signal.

The activity of the dnaI gene is defined as the expression of an RNA encoding a *S. aureus* DnaI polypeptide according to the invention.

As used herein, the term "polynucleotide encoding a polypeptide" or equivalent language encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of *S. aureus* DnaI protein having an amino acid sequence set out in Fig. 1, SEQ ID NO: 2. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to

RNA editing or genomic DNA reorganization) together with additional regions that also may contain coding and/or non-coding sequences.

As used herein, the term "dnaI gene" is meant to encompass a polynucleotide encoding a *S. aureus* DnaI polypeptide. Any additional nucleotide sequences necessary to direct transcription of RNA encoding a *S. aureus* DnaI polypeptide, either in a cell or in vitro, will be termed "regulatory sequences", which include but are not limited to transcriptional promoters and enhancers, and transcription terminators.

As used herein, the term "ORF 104" or "phage 77 ORF 104" or "77ORF104" encompasses a polynucleotide having the sequence provided in Fig. 4 (SEQ ID No: 4), which encodes a gene product known as the 77 ORF 104 gene product.

As used herein, the term "polynucleotide(s)" generally refers to any polyribonucleotide or poly-deoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotide(s)" include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions or single-, double- and triple-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded, or triple-stranded regions, or a mixture of single- and double-stranded regions. In addition, "polynucleotide" as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the

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molecules of a triple-helical region often is an oligonucleotide. As used herein, the term "polynucleotide(s)"also includes DNAs or RNAs as described above that contain one or more modified bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "polynucleotide(s)" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein. It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term "polynucleotide(s)" as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including, for example, simple and complex cells. "Polynucleotide(s)" also embraces short polynucleotides often referred to as oligonucleotide(s).

As used herein, the term "polypeptide(s)" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds. "Polypeptide(s)" refers to both short chains, commonly referred to as peptides, oligopeptides and oligomers and to longer chains generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptide(s)" include those modified either by natural processes, such as processing and other post-translational modifications, but also by chemical modification techniques. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature, and they are well known to those of skill in the art. It will be appreciated that the same type of modification

may be present in the same or varying degree at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains, and the amino or carboxyl termini. Modifications include, for example, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation, selenoylation, sulfation and transfer-RNA mediated addition of amino acids to proteins, such as arginylation, and ubiquitination. See, for instance: PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York (1983); Seifter et al., Meth. Enzymol. 182:626-646 (1990); and Rattan et al., Protein Synthesis: Posttranslational Modifications and Aging, Ann. N.Y. Acad. Sci. 663: 48-62(1992). Polypeptides may be branched or cyclic, with or without branching. Cyclic, branched and branched circular polypeptides may result from post-translational natural processes and may be made by entirely synthetic methods, as well.

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As used herein, the term "variant(s)" refers to a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide, respectively, but retains one or more of the biological activities of DnaI as described herein. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, and truncations in the polypeptide encoded by the reference sequence, or in the formation of fusion proteins, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. The present invention also includes variants of each of the polypeptides of the invention, that is polypeptides that vary from the referents by conservative amino acid substitutions whereby a residue is substituted by another with like characteristics. Typically, such substitutions are among Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which 1-10, 1-5, 1-3, 2-3, or 1 amino acid or amino acids are substituted, deleted, or added in any combination. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of

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polynucleotides and polypeptides may be made by mutagenesis techniques, by direct synthesis, and by other recombinant methods known to skilled artisans.

As uses herein, the term "fragment", when used in reference to a polypeptide, is a variant polypeptide having an amino acid sequence that is entirely the same as part but not all of the amino acid sequence of DnaI polypeptide according to the invention. As with *S. aureus* DnaI polypeptides, fragments may be "free-standing" ("consisting of"), or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region in a single larger polypeptide.

The term "isolated", when used in reference to a nucleic acid means that a naturally occurring sequence has been removed from its normal cellular (*e.g.*, chromosomal) environment or is synthesized in a non-natural environment (*e.g.*, artificially synthesized). Thus, the sequence may be in a cell-free solution or placed in a different cellular environment. The term does not imply that the sequence is the only nucleotide chain present, but that it is essentially free (about 90-95% pure at least) of non-nucleotide material naturally associated with it, and thus is distinguished from isolated chromosomes.

The term "enriched", when used in reference to a polynucleotide means that the specific DNA or RNA sequence constitutes a significantly higher fraction (2-5 fold) of the total DNA or RNA present in the cells or solution of interest than in normal or diseased cells or in cells from which the sequence was originally taken. This could be caused by a person by preferential reduction in the amount of other DNA or RNA present, or by a preferential increase in the amount of the specific DNA or RNA sequence, or by a combination of the two. However, it

should be noted that enriched does not imply that there are no other DNA or RNA sequences present, just that the relative amount of the sequence of interest has been significantly increased.

As used herein, the term "significantly higher fraction" indicates that the level of enrichment is useful to the person making such an enrichment and indicates an increase enrichment relative to other nucleic acids of at least about 2-fold, or 5- to 10-fold or even more. The term also does not imply that there is no DNA or RNA from other sources. The other source DNA may, for example, comprise DNA from a yeast or bacterial genome, or a cloning vector such as pUC19. This term distinguishes from naturally occurring events, such as viral infection, or tumor type growths, in which the level of one mRNA may be naturally increased relative to other species of mRNA. That is, the term is meant to cover only those situations in which a person has intervened to elevate the proportion of the desired nucleic acid.

As used herein, the term "purified" in reference to nucleic acid does not require absolute purity (such as a homogeneous preparation). Instead, it represents an indication that the sequence is relatively more pure than in the natural environment (compared to the natural level, this level should be at least 2-5 fold greater, *e.g.*, in terms of mg/mL). Individual clones isolated from a genomic or cDNA library may be purified to electrophoretic homogeneity. The claimed DNA molecules obtained from these clones could be obtained directly from total DNA or from total RNA. cDNA clones are not naturally occurring, but rather are preferably obtained via manipulation of a partially purified naturally occurring substance (messenger RNA). The construction of a cDNA library from mRNA involves the creation of a synthetic substance (cDNA) and pure individual cDNA clones can be isolated from the synthetic library by clonal

selection of the cells carrying the cDNA library. Thus, the process which includes the construction of a cDNA library from mRNA and isolation of distinct cDNA clones yields an approximately 10^6-fold purification of the native message over its proportion in naturally occurring cells. Thus, purification of at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated. A genomic library can be used in the same way and yields the same approximate levels of purification.

The terms "isolated", "enriched", and "purified" used with respect to nucleic acids, above, may similarly be used to denote the relative purity and abundance of polypeptides. These, too, may be stored in, grown in, screened in, and selected from libraries using biochemical techniques familiar in the art. Such polypeptides may be natural, synthetic or chimeric and may be extracted using any of a variety of methods, such as antibody immunoprecipitation, other "tagging" techniques, conventional chromatography and/or electrophoretic methods. Some of the above utilize the corresponding nucleic acid sequence.

As used herein, the term "complement" when used in reference to a given polynucleotide sequence refers to a sequence of nucleotides which can form a double-stranded heteroduplex in which every nucleotide in the sequence of nucleotides is base-paired by hydrogen bonding to a nucleotide opposite it in the heteroduplex with the given polynucleotide sequence. The term may refer to a DNA or an RNA sequence that is the complement of another RNA or DNA sequence. As used herein, the term "hybridizes" refers to the formation of a hydrogen-bonded heteroduplex between two nucleic acid molecules. Generally, a given nucleic acid molecule will

hybridize with its complement, or with a molecule that is sufficiently complementary to the given molecule to permit formation of a hydrogen-bonded heteroduplex between the two molecules.

As used herein, the term "probe" refers to a polynucleotide of at least 15 nucleotides (nt), 20 nt, 30 nt, 40 nt, 50 nt, 75 nt, 100 nt, 200 nt, 500 nt, 1000 nt, and even up to 5000 to 10,000 nt in length.

"Identity" and "similarity," as used herein and as known in the art, are relationships between two or more polypeptide sequences or two or more polynucleotide sequences, as the case may be, as determined by comparing the sequences.

Amino acid or nucleotide sequence "identity" and "similarity" are determined from an optimal global alignment between the two sequences being compared. An optimal global alignment is achieved using, for example, the Needleman - Wunsch algorithm (Needleman and Wunsch, 1970, J. Mol. Biol. 48:443-453). "Identity" means that an amino acid or nucleotide at a particular position in a first polypeptide or polynucleotide is identical to a corresponding amino acid or nucleotide in a second polypeptide or polynucleotide that is in an optimal global alignment with the first polypeptide or polynucleotide. In contrast to identity, "similarity" encompasses amino acids that are conservative substitutions. A "conservative" substitution is any substitution that has a positive score in the blosum62 substitution matrix (Hentikoff and Hentikoff, 1992, Proc. Natl. Acad. Sci. USA 89: 10915-10919). By the statement "sequence A is n% similar to sequence B" is meant that n% of the positions of an optimal global alignment between sequences A and B consists of conservative substitutions. By the statement "sequence

A is n% identical to sequence B" is meant that n% of the positions of an optimal global alignment between sequences A and B consists of identical residues or nucleotides. Optimal global alignments in this disclosure used the following parameters in the Needleman-Wunsch alignment algorithm:

For polypeptides:

Substitution matrix: blosum62.

Gap scoring function: -A -B*LG, where A=11 (the gap penalty), B=1 (the gap length penalty) and LG is the length of the gap.

For nucleotide sequences:

Substitution matrix: 10 for matches, 0 for mismatches.

Gap scoring function: -A -B*LG where A=50 (the gap penalty), B=3 (the gap length penalty) and LG is the length of the gap.

Typical conservative substitutions are among Met, Val, Leu and Ile; among Ser and Thr; among the residues Asp, Glu and Asn; among the residues Gln, Lys and Arg; or aromatic residues Phe and Tyr. In calculating the degree (most often as a percentage) of similarity between two polypeptide sequences, one considers the number of positions at which identity or similarity is observed between corresponding amino acid residues in the two polypeptide sequences in relation to the entire lengths of the two molecules being compared.

As used herein, the term "antibody" is meant to encompass constructions using the binding (variable) region of such an antibody, and other antibody modifications. Thus, an antibody useful in the invention may comprise a whole antibody, an antibody fragment, a

polyfunctional antibody aggregate, or in general a substance comprising one or more specific binding sites from an antibody. The antibody fragment may be a fragment such as an Fv, Fab or $F(ab')_2$ fragment or a derivative thereof, such as a single chain Fv fragment. The antibody or antibody fragment may be non-recombinant, recombinant or humanized. The antibody may be of an immunoglobulin isotype, e.g., IgG, IgM, and so forth. In addition, an aggregate, polymer, derivative and conjugate of an immunoglobulin or a fragment thereof can be used where appropriate. Neutralizing antibodies are especially useful according to the invention for diagnostics, therapeutics and methods of drug screening and drug design.

As used herein, the term "specific for an epitope present on a *S. aureus* DnaI polypeptide", when used in reference to an antibody, means that the antibody recognizes and binds an antigenic determinant present on a *S. aureus* DnaI polypeptide according to the invention.

As used herein, the term "antigenically equivalent derivative(s)" encompasses a polypeptide, polynucleotide, or the equivalent of either which will be specifically recognized by certain antibodies which, when raised to the protein, polypeptide or polynucleotide according to the invention, interferes with the immediate physical interaction between pathogen and mammalian host.

As used herein, the term "essential", when used in connection with a gene or gene product, means that the host cannot survive without, or is significantly growth compromised, in the absence or depletion of functional product. An "essential gene" is thus one that encodes a product that is beneficial, or preferably necessary, for cellular growth *in vitro* in a medium

appropriate for growth of a strain having a wild-type allele corresponding to the particular gene in question. Therefore, if an essential gene is inactivated or inhibited, that cell will grow significantly more slowly than a wild-type strain or even not at all. Preferably, growth of a strain in which such a gene has been inactivated will be less than 20%, more preferably less than 10%, most preferably less than 5% of the growth rate of the wild-type, or the rate will be zero, in the growth medium. Preferably, in the absence of activity provided by a product of the gene, the cell will not grow at all or will be non-viable, at least under culture conditions similar to normal *in vivo* growth conditions. For example, absence of the biological activity of certain enzymes involved in bacterial cell wall synthesis can result in the lysis of cells under normal osmotic conditions, even though protoplasts can be maintained under controlled osmotic conditions. Preferably, but not necessarily, if such a gene is inhibited, e.g., with an antibacterial agent or a phage product, the growth rate of the inhibited bacteria will be less than 50%, more preferably less than 30%, still more preferably less than 20%, and most preferably less than 10% of the growth rate of the uninhibited bacteria. As recognized by those skilled in the art, the degree of growth inhibition will generally depend upon the concentration of the inhibitory agent. In the context of the invention, essential genes are generally the preferred targets of antimicrobial agents. Essential genes can encode "target" molecules directly or can encode a product involved in the production, modification, or maintenance of a target molecule.

As used herein, "target" refers to a biomolecule or complex of biomolecules that can be acted on by an exogenous agent or compound, thereby modulating, preferably inhibiting, growth

or viability of a bacterial cell. A target may be a nucleic acid sequence or molecule, or a polypeptide or a region of a polypeptide.

As used herein, the term "signal that is generated by activation or inhibition of a *S. aureus* DnaI polypeptide" refers to the measurable indicator of DnaI activity in an assay of DnaI activity. For example, ^{3}H-thymidine DNA incorporation, plasmid replication, helicase loading, or simply signal resulting for binding of 77ORF104 to a DnaI polypeptide.

As used herein, the term "standard", used in reference to polypeptide activity, means the amount of activity observed or detected (directly or indirectly) in a given assay performed in the absence of a candidate compound. A "standard" serves as a reference to determine the effect, positive or negative, of a candidate compound on polypeptide activity.

A "candidate compound" as used herein, is any compound with a potential to modulate the expression or activity of a *S. aureus* DnaI polypeptide.

As used herein, the term "increase in activity" refers to an enhanced level of measurable activity of a polypeptide in a given assay in the presence of a candidate compound relative to the measurable level of activity in the absence of a candidate compound. Activity is considered increased according to the invention if it is at least 10% greater, 20% greater, 50% greater, 75% greater, 100% greater or more, up to 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, 100-fold or more than in the absence of a candidate compound.

As used herein, the term "decrease in activity" refers to a reduced level of measurable activity of a polypeptide in a given assay in the presence of a candidate compound relative to the measurable level of activity in the absence of a candidate compound. Activity is considered

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decreased according to the invention if it is at least 10% less, preferably 15% less, 20% less, 50% less, 75% less, or even 100% less (i.e., no activity) than that observed in the absence of a candidate compound.

As used herein, the term "conditions that permit their interaction", when used in reference to a *S. aureus* DnaI polypeptide and a candidate compound means that the two entities are placed together, whether both in solution or with one immobilized or restricted in some way and the other in solution, wherein the parameters (e.g., salt, detergent, protein or candidate compound concentration, temperature, and redox potential, among others) of the solution are such that the *S. aureus* DnaI polypeptide and the candidate compound may physically associate. Conditions that permit protein:candidate interaction include, for example, the conditions described herein for Phage display, Surface Plasmon Resonance and FRET assays.

As used herein, the term "detectable change in a measurable parameter of DnaI" refers to an alteration in a quantifiable characteristic of a *S. aureus* DnaI polypeptide.

As used herein, the term "agonist" refers to an agent or compound that enhances or increases the activity of a *S. aureus* DnaI polypeptide or polynucleotide. An agonist may be directly active on a *S. aureus* DnaI polypeptide or polynucleotide, or it may be active on one or more constituents in a pathway that leads to enhanced or increased activity of a *S. aureus* DnaI polypeptide or polynucleotide.

As used herein, the term "antagonist" refers to an agent or compound that reduces or decreases the activity of a *S. aureus* DnaI polypeptide or polynucleotide. An antagonist may be directly active on a *S. aureus* DnaI polypeptide or polynucleotide, or it may be active on one or

more constituents in a pathway that leads to reduced or decreased activity of a *S. aureus* DnaI polypeptide or polynucleotide.

As used herein, the term "antibacterial agent" or "antibacterial compound" refers to an agent or compound that has a bacteriocidal or bacteriostatic effect on one or more bacterial strains, preferably such an agent or compound is bacteriocidal or bacteriostatic on at least *S. aureus*.

As used herein, the term "synthesizing" refers to a process of chemically synthesizing a compound.

As used in the context of treating a bacterial infection a "therapeutically effective amount", "pharmaceutically effective amount" or "amount sufficient to provide a therapeutic effect" indicates an amount of an antibacterial agent which has a therapeutic effect. This generally refers to the inhibition, to some extent, of the normal cellular functioning of bacterial cells required for continued bacterial infection. Further, as used herein, a therapeutically effective amount means an amount of an antibacterial agent that produces the desired therapeutic effect as judged by clinical trial results and/or animal models. This amount can be routinely determined by one skilled in the art and will vary depending on several factors, such as the particular bacterial strain involved and the particular antibacterial agent used. In the same context, an "amount sufficient to reduce adhesion" of a bacterium to a tissue or tissue surface indicates an amount of an antibacterial agent that is effective for prophylactically preventing or reducing the extent of bacterial infection of the given tissue or tissue surface.

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As used herein, a "tissue" refers to an aggregation of cells of one or more cell types which together perform one or more specific functions in an organism. As used herein, a "tissue surface" refers to that portion of a tissue that forms a boundary between a given tissue and other tissues or the surroundings of the tissue. A tissue surface may refer to an external surface of an animal, for example the skin or cornea, or, alternatively, the term may refer to a surface that is either internal, for example, the lining of the gut, or to a surface that is exposed to the outside surroundings of the animal only as the result of an injury or a surgical procedure.

As used herein, the term "measuring the binding of a candidate compound" refers to the use of an assay permitting the quantitation of the amount of a candidate compound physically associated with a *S. aureus* DnaI polypeptide.

As used herein, the term "directly or indirectly detectably labeled" refers to the attachment of a moiety to a candidate compound that renders the candidate compound either directly detectable (e.g., an isotope or a fluorophore) or indirectly detectable (e.g., an enzyme activity, allowing detection in the presence of an appropriate substrate, or a specific antigen or other marker allowing detection by addition of an antibody or other specific indicator).

As used herein, the term "small molecule" refers to compounds having molecular mass of less than 3000 Daltons, preferably less than 2000 or 1500, still more preferably less than 1000, and most preferably less than 600 Daltons. Preferably but not necessarily, a small molecule is not an oligopeptide.

As used herein, the term "mimetic" refers to a compound that can be natural, synthetic, or chimeric and is structurally and functionally related to a reference compound. In terms of the

present invention, a "peptidomimetic," for example, is a non-peptide compound that mimics the activity-related aspects of the 3-dimensional structure of a peptide or polypeptide, for example a compound that mimics the structure of a peptide or active portion of a phage- or bacterial ORF-encoded polypeptide.

As used herein, the term "bacteriophage inhibitor protein" refers to a protein encoded by a bacteriophage nucleic acid sequence, which inhibits bacterial function in a host bacterium. Thus, it is a bacteria-inhibiting phage product. The term "bacteriophage inhibitor protein" encompasses a fragment, derivative, or active portion of a bacteriophage inhibitor protein.

As used herein, the term "active portion" refers to an epitope, a catalytic or regulatory domain, or a fragment of a bacteriophage inhibitor protein that is responsible for, or a significant factor in, bacterial target inhibition. The active portion preferably may be removed from its contiguous sequences and, in isolation, still effect inhibition.

As used herein, the term "treating a bacterial infection" refers to a process whereby the growth and/or metabolic activity of a bacterium or bacterial population in a host, preferably a mammal, more preferably a human, is inhibited or ablated.

As used herein, the term "bacterium" refers to a single bacterial strain and includes a single cell and a plurality or population of cells of that strain unless clearly indicated to the contrary. In reference to bacteria or bacteriophage, the term "strain" refers to bacteria or phage having a particular genetic content. The genetic content includes genomic content as well as recombinant vectors. Thus, for example, two otherwise identical bacterial cells would represent different strains if each contained a vector, *e.g.*, a plasmid, with different inserts.

As used herein, the term "diagnosing" refers to the identification of an organism or strain of an organism responsible for a bacterial infection.

As used herein, the term "infection with *Staphylococcus aureus*" refers to the presence, growth or proliferation of cells of a *S. aureus* strain within, or on a surface of, an animal, such as a mammal, preferably a human.

As used herein, the term "bacteriophage 77 ORF 104-encoded polypeptide" refers to a polypeptide encoded by SEQ ID NO: 4 or to a fragment or derivative thereof encompassing an active portion of a bacteriophage 77 ORF 104-encoded polypeptide of sequence disclosed in SEQ ID NO: 5.

As used herein, the term "DnaC" refers to a polypeptide of SEQ ID NO: 9, including that encoded by a polynucleotide of SEQ ID NO: 7 or to a fragment or derivative of such polypeptide encompassing an active portion of *S. aureus* DnaC. In this context, an active portion of *S. aureus* DnaC refers to that fragment or portion of *S. aureus* DnaC that interacts with or is part of a complex including *S. aureus* DnaI or a fragment or derivative of *S. aureus* DnaI.

As used herein, the term "polypeptide complex" refers to a combination of two or more polypeptides in a physical association with each other. It is preferred that such a physical association be required for some aspect of the activity of one or more of the polypeptides in such a polypeptide complex.

As used herein, the term "physical association" refers to an interaction between two moieties involving contact between the two moieties.

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As used herein, the term "bodily material(s)" means any material derived from an individual or from an organism infecting, infesting or inhabiting an individual, including but not limited to, cells, tissues and waste, such as, bone, blood, serum, cerebrospinal fluid, semen, saliva, muscle, cartilage, organ tissue, skin, urine, stool or autopsy materials.

As used herein, the term "disease(s)" means any disease caused by or related to infection by a bacterium, including, for example, otitis media, conjunctivitis, pneumonia, bacteremia, meningitis, sinusitis, pleural empyema and endocarditis, and most particularly meningitis, such as for example infection of cerebrospinal fluid.

As used herein, the term "fusion protein(s)" refers to a protein encoded by a gene comprising amino acid coding sequences from two or more separate proteins fused in frame such that the protein comprises fused amino acid sequences from the separate proteins.

As used herein, the term "host cell(s)" is a cell which has been transformed or transfected, or is capable of transformation or transfection by an exogenous polynucleotide sequence.

As used herein, the term "immunologically equivalent derivative(s)" encompasses a polypeptide, polynucleotide, or the equivalent of either which when used in a suitable formulation to raise antibodies in a vertebrate, results in antibodies that act to interfere with the immediate physical interaction between pathogen and mammalian host.

As used herein, the term "immunospecific" means that characteristic of an antibody whereby it possesses substantially greater affinity for the polypeptides of the invention or the

polynucleotides of the invention than its affinity for other related polypeptides or polynucleotides respectively, particularly those polypeptides and polynucleotides in the prior art.

As used herein, the term "individual(s)" means a multicellular eukaryote, including, but not limited to a metazoan, a mammal, an ovid, a bovid, a simian, a primate, and a human.

As used herein, the term "Organism(s)" means a (i) prokaryote, including but not limited to, a member of the genus Streptococcus, Staphylococcus, Bordetella, Corynebacterium, Mycobacterium, Neisseria, Haemophilus, Actinomycetes, Streptomycetes, Nocardia, Enterobacter, Yersinia, Fancisella, Pasturella, Moraxella, Acinetobacter, Erysipelothrix, Branhamella, Actinobacillus, Streptobacillus, Listeria, Calymmatobacterium, Brucella, Bacillus, Clostridium, Treponema, Escherichia, Salmonella, Kleibsiella, Vibrio, Proteus, Erwinia, Borrelia, Leptospira, Spirillum, Campylobacter, Shigella, Legionella, Pseudomonas, Aeromonas, Rickettsia, Chlamydia, Borrelia and Mycoplasma, and further including, but not limited to, a member of the species or group, Group A Streptococcus, Group B Streptococcus, Group C Streptococcus, Group D Streptococcus, Group G Streptococcus, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus faecalis, Streptococcus faecium, Streptococcus durans, Neisseria gonorrheae, Neisseria meningitidis, Staphylococcus aureus, Staphylococcus epidermidis, Corynebacterium diptheriae, Gardnerella vaginalis, Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium ulcerans, Mycobacterium leprae, Actinomyctes israelii, Listeria monocytogenes, Bordetella pertusis, Bordatella parapertusis, Bordetella bronchiseptica, Escherichia coli, Shigella dysenteriae, Haemophilus influenzae, Haemophilus aegyptius, Haemophilus parainfluenzae, Haemophilus ducreyi,

Bordetella, Salmonella typhi, Citrobacter freundii, Proteus mirabilis, Proteus vulgaris, Yersinia pestis, Kleibsiella pneumoniae, Serratia marcessens, Serratia liquefaciens, Vibrio cholera, Shigella dysenterii, Shigella flexneri, Pseudomonas aeruginosa, Franscisella tularensis, Brucella abortis, Bacillus anthracis, Bacillus cereus, Clostridium perfringens, Clostridium tetani, Clostridium botulinum, Treponema pallidum, Rickettsia rickettsii and Chlamydia trachomitis, (ii) an archaeon, including but not limited to Archaebacter, and (iii) a unicellular or filamenous eukaryote, including but not limited to, a protozoan, a fungus, a member of the genus Saccharomyces, Kluveromyces, or Candida, and a member of the species Saccharomyces ceriviseae, Kluveromyces lactis, or Candida albicans.

As used herein, the term "recombinant expression system(s)" refers to a system in which vectors comprising sequences encoding polypeptides of the invention or portions thereof, or polynucleotides of the invention are introduced or transformed into a host cell or host cell lysate for the production of the polynucleotides and polypeptides of the invention.

As used herein, the term "artificially synthesized" when used in reference to a peptide, polypeptide or polynucleotide means that the amino acid or nucleotide subunits were chemically joined in vitro without the use of cells or polymerizing enzymes. The chemistry of polynucleotide and peptide synthesis is well known in the art.

In addition to the standard single and triple letter representations for amino acids, the term "X" or "Xaa" may also be used in describing certain polypeptides of the invention. "X" and "Xaa" mean that any of the twenty naturally occurring amino acids may appear at such a designated position in the polypeptide sequence.

As used herein, the term "specifically binding" in the context of the interaction of two polypeptides means that the two polypeptides physically interact via discrete regions or domains on the polypeptides, wherein the interaction is dependent upon the amino acid sequences of the interacting domains. Generally, the equilibrium binding concentration of a polypeptide that specifically binds another is in the range of about 1 μM or lower, preferably 100 nM or lower, 10 nM or lower, 1 nM or lower, 100 pM or lower, and even 10 pM or lower.

As used herein, the term "decrease in the binding" refers to a drop in the signal that is generated by the physical association between two polypeptides under one set of conditions relative to the signal under another set of reference conditions. The signal is decreased if it is at least 10% lower than the level under reference conditions, and preferably 20%, 40%, 50%, 75%, 90%, 95% or even as much as 100% lower (i.e., no detectable interaction).

As used herein, the term "detectable marker", when used in the context of a yeast two-hybrid assay, refers to a polypeptide that confers a trait upon a cell expressing that polypeptide that signals the presence or amount of that polypeptide expressed. Detectable markers are encoded on plasmids that may exist episomally or may be integrated into the genome of a host cell. Detectable markers include, but are not limited to, polypeptides encoding enzymes allowing colorimetric or fluorescent detection (e.g., E. coli LacZ, which catalyzes the conversion of the substrate analog X-gal to generate a blue color), polypeptides encoding enzymes conferring antibiotic resistance, and polypeptides encoding enzymes conferring the ability of a yeast strain to grow on medium lacking a given component (i.e., critical for the relief of auxotrophy).

As used herein, the term "results in the expression of a detectable marker" means that the interaction of factors necessary to permit the expression of a detectable marker (e.g., two-hybrid transactivation domain and DNA binding domain fusion proteins) causes the transactivation and translation of detectable levels of a detectable marker. A "detectable level" is that level of expression that can be differentiated from background expression occurring in the substantial absence of one or more factors or conditions necessary for marker expression. Detectable levels will vary depending upon the nature of the detectable marker, but will generally consist of levels at least about 10% or more greater than the background level of a given marker.

As used herein, the term "decrease in the expression" refers to a drop in the expression of a detectable marker under one set of conditions relative to the expression under another set of reference conditions. The expression of a detectable marker is decreased if it is at least 10% lower than the level under reference conditions, and preferably 20%, 40%, 50%, 75%, 90%, 95% or even as much as 100% lower (i.e., not expressed).

How to Identify a *S. aureus* dnaI sequence:

Using methodology described in detail in Example 1 and 2, a *S. aureus* polypeptide that specifically bound the bacterial growth inhibitory 77 phage ORF 104 protein was isolated. Briefly, the 77ORF104 protein was used as a ligand in an affinity chromatography binding step with *S. aureus* protein extract. The selected *S. aureus* interacting polypeptide was purified and further analyzed by tryptic digestion and mass spectrometry. The sequence of a tryptic peptide of the *S. aureus* polypeptide, GHVPENVTDNDR (SEQ ID NO: 10), was used to BLAST search the *S. aureus* nucleotide sequence in the University of Oklahoma *S. aureus* genomic database at

http://www.genome.ou.edu/staph.html. One sequence contig of 4850 nucleotides in length (Contig 981), when converted into amino acid sequence, contained within it the similar amino acid sequence GHVPELYVDNNR (SEQ ID NO: 11; Fig. 5). This tentative identification of the candidate protein was then confirmed upon *in silico* tryptic digestion of the open reading frame found in the contig (Fig. 5). The obtained PSD/CID spectra for tryptic peptides with monoisotopic MH+ masses of 1351.8, 1412.7, and 1617.8 Da were similar to the predicted PSD/CID fragmentation patterns of the tryptic peptides with monoisotopic MH+ masses of 1351.8 and 1617.8 Da found in the contig's +3 open reading frame (Fig. 5).

Comparison of the ORF of the *S. aureus* contig that encodes a tryptic peptide similar to that identified in the *S. aureus* phage 77 ORF 104 binding studies with all other sequences in the public domain databases revealed that the ORF is related to the DnaI protein from *Bacillus subtilis* (Table 1) a protein implicated in chromosome replication. No other significant similarity was found with any other protein in publicly accessible databases. The degree of relatedness of the identified ORF to the *B. subtilis* DnaI protein shows 41% identity and 63% similarity (Table 1).

Many genes of *B. subtilis* involved in DNA replication have been identified through the isolation of thermosensitive mutants. One of these, dnaI2, affected an unknown step of chromosome replication at the restrictive temperature (Karamata, D. and Gross, J.D. (1970) Mol. Gene. Genet. **108,** 277-287). The gene was mapped around 250° on the *B subtilis* chromosome and resides immediately downstream of the dnaB gene on the *B. subtilis* chromosome (Bruand, C. and Ehrlich, S.D. (1995) Microbiology **141,** 1199-1200). The dnaI2 mutation has been characterized and resides within the dnaI gene and consists of a G to A substitution at nucleotide position 922 (Fig. 1; SEQ ID NO: 1) resulting in a glycine to glutamate change at position 307 (Fig. 1; SEQ ID NO: 2) (Bruand, C. and Ehrlich, S.D. (1995) Microbiology **141,** 1199-1200).

DnaC has been genetically identified to be the major component DNA helicase of chromosome replication (Sakamoto, Y., Nakai, S., Moriya, S., Yoshikawa, H., and Ogasawara, N. (1995) Microbiology **141,** 641-644) and is thought to unwind duplex DNA progressively and allow for binding of the DNA polymerase III holoenzyme necessary for priming and DNA synthesis. One possible function of DnaI is as a helicase loader, being responsible for transferring DnaC helicase to the oriC. The product of the dnaC and dnaI genes are required for chromosome replication and are all essential for DNA replication in *B. subtilis* (Ceglowski, P., Lurz, R., Alonso, J.C.J. (1993) Mol. Biol. **236,** 1324-1340).

Databases were searched for *S. aureus* genes which may be related to the *B. subtilis* dnaC gene. Utilizing the *B. subtilis* amino acid sequence for DnaC (Accession Number P37469), a BLAST search was performed of the Staphylococcus database at http://www.tigr.org and revealed the presence of an ORF within the *S. aureus* genome encoding a related protein. The nucleotide sequence and corresponding protein sequence are presented in Fig. 6A (SEQ ID NO: 7) and Fig. 6B (SEQ ID NO: 9), respectively.

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Identification of the surface of interaction on DnaI

This invention relates, in part, to a specific interaction between a growth-inhibitory protein encoded by the *Staphylococcus aureus* bacteriophage 77 genome and an essential *S. aureus* protein. This interaction forms the basis for drug screening assays. More specifically, the invention relates to the interacting regions of the protein encoded by the *S. aureus* bacteriophage 77 and the *S. aureus* DnaI proteins, forming the basis for screening assays. The invention provides a method for the identification of DnaI polypeptide fragments that are involved in said interaction between DnaI and ORF 104 from bacteriophage 77. Several approaches and techniques known to those skilled in the art can be used to identify and to characterize fragments of the DnaI interacting with 77 ORF 104. These fragments may include, for example, truncation polypeptides having a portion of an amino acid sequence of the proteins, or variants thereof, such as a continuous series of residues that includes an amino- and/or carboxyl-terminal amino acid sequence for DnaI.

A) Affinity Chromatography

Partial proteolysis of proteins in solution is one method to delineate the domain boundaries in multi-domain proteins. By subjecting proteins to limited digestion, the most accessible cleavage sites are preferentially hydrolyzed. These cleavage sites preferentially reside in less structured regions which include loops and highly mobile areas typical of the joining amino acids between highly structured domains. For this analysis, a purified recombinant DnaI polypeptide (including a fragment of DnaI either purified from a previous protease digestion or expressed from a recombinant nucleic acid vector as a fragment) can be subjected to partial proteolysis. The proteolysis can be performed with low concentrations of proteases, including, but not limited to trypsin, chymotrypsin, endoproteinase Glu-C, and Asp-N with a DnaI

polypetide in solution, resulting in the generation of defined proteolytic products as observed by SDS-PAGE. An acceptable concentration and reaction time is defined by the near complete conversion of the full-length protein to stable proteolytic products. The partial proteolytic fragments are then subjected to affinity chromatography with immobilized 77 ORF 104 to determine the region of the DnaI polypeptide containing the 77 ORF 104 binding site. Interacting domains are identified by mass spectrometry to determine the masses of both the intact fragment and the series of fragments from a tryptic digest to identify the amino acid residues contained within the partial proteolytic fragment. Using both sets of data, the amino acid sequence of the partial proteolytic fragment can be precisely determined.

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B) Yeast two-hybrid analysis

The interaction between 77 ORF 104 and portions of the DnaI polypeptide can also be assessed *in vivo* using the yeast two hybrid system. To do this, bacteriophage 77 ORF 104 is fused to the DNA binding domain of the yeast transcriptional transactivator Gal4, and different portions of the DnaI polypeptide are fused to the carboxyl terminus of the Gal4 activation domain. The two plasmids bearing such constructs can be introduced sequentially, or in combination, into a yeast cell line, for example AH109 (Clontech Laboratories), previously engineered to contain chromosomally-integrated copies of *E. coli lacZ* and the selectable *HIS3* and *ADE2* genes. The *lacZ*, *HIS,* and *ADE2* reporter genes, each driven by a promoter containing Gal4 binding sites, are used for measuring protein-protein interactions. If the two recombinant proteins interact within the yeast cell, the resulting protein:protein complex activates transcription from promoters containing Gal4 binding sites. Expression of *HIS3,* and *ADE2* genes is manifested by relief of histidine and adenine auxotrophy. As described in the examples

below, full length DnaI, as well as DnaI fragments, was found to interact with bacteriophage 77 ORF 104 fusion polypeptides using this system.

Further elucidation of the bacteriophage 77 ORF 104 interacting domain of DnaI can be carried out by first subjecting the full length DnaI polypeptide to deletional mutagenesis, the methods of which are known to those of skill in the art. The mutated DnaI polypeptides can then be subjected to yeast two hybrid analysis as described above, to further narrow those amino acid sequences or polypeptide fragments, for example, those within SEQ ID NO: 16, that are required for the binding of DnaI to bacteriophage 77 ORF 104.

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S. aureus DnaI polypeptides

In one aspect of the invention there are provided polypeptides of *S. aureus* referred to herein as "DnaI" and "DnaI polypeptides" as well as biologically, diagnostically, prophylactically, clinically or therapeutically useful variants thereof, and compositions comprising the same.

Among the particularly preferred embodiments of the invention are variants of *S. aureus* DnaI polypeptides encoded by naturally occurring alleles of the dnaI gene. The present invention provides for an isolated polypeptide which comprises or consists of: (a) an amino acid sequence which has at least 50% identity, preferably at least 80% identity, more preferably at least 90%, yet more preferably at least 95%, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:2 over the entire length of SEQ ID NO: 2 or b) an amino acid sequence that has at least 70% similarity, at least 80% similarity, at least 90% similarity, at least 95% similarity, at least 97-99% similarity or even 100% similarity over the entire length of SEQ ID NO: 2.

The polypeptides of the invention include a polypeptide of Fig. 1 (SEQ ID NO: 2) (in particular the mature polypeptide) as well as polypeptides and fragments, particularly those

which have the biological activity of DnaI, and also those which have at least 50% identity over 20, 40, 50 or more amino acids to a polypeptide of SEQ ID NO: 2 or the relevant portion, preferably at least 60%, 70%, or 80% identity, more preferably at least 90% identity to a polypeptide of SEQ ID NO: 2 and more preferably at least 90% identity to a polypeptide of SEQ ID NO: 2 and still more preferably at least 95% identity to a polypeptide of SEQ ID NO: 2 and yet still more preferably at least 99% identity to a polypeptide of SEQ ID NO: 2.

The polypeptides of the invention also include a polypeptide or protein fragment that has at least 60%, 70%, 80% or 90% similarity, 95% similarity or even 97-99% similarity over 10, 20, 25, 30 or more amino acids to a polypeptide of SEQ ID NO: 2. It is preferred that a polypeptide of the invention has at least 60% similarity to a polypeptide of SEQ ID NO: 2 over at least 20 amino acids.

It is most preferred that a polypeptide of the invention is derived from S. aureus, however, it may be obtained from other organisms of the same taxonomic genus. A polypeptide of the invention may also be obtained, for example, from organisms of the same taxonomic family or order.

Fragments of DnaI also are included in the invention. These fragments may include, for example, truncation polypeptides having a portion of an amino acid sequence of Fig. 1 (SEQ ID NO: 2), or variants thereof, such as a continuous series of residues that includes an amino- and/or carboxyl-terminal amino acid sequence. Degradation forms of the polypeptides of the invention produced by or in a host cell, particularly *S. aureus*, are also preferred. Further preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix-forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions,

alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Fragments of DnaI may be expressed as fusion proteins with other proteins or protein fragments.

Preferred fragments also include an isolated polypeptide comprising an amino acid sequence having at least 20, 30, 40, 50, or 100 contiguous amino acids from the amino acid sequence of SEQ ID NO: 2.

Also preferred are biologically "active" fragments which are those fragments that mediate activities of *S. aureus* DnaI, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those fragments that are antigenic or immunogenic in an animal, especially in a human. Particularly preferred are fragments comprising domains that confer a function essential for viability of *S. aureus*.

Fragments of the polypeptides of the invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, these variants may be employed as intermediates for producing the full-length polypeptides of the invention.

S. aureus Polynucleotides

It is an object of the invention to provide polynucleotides that encode DnaI polypeptides, particularly polynucleotides that encode the polypeptide herein designated *S. aureus* DnaI.

In one aspect of the invention a polynucleotide is provided that comprises a region encoding a *S. aureus* DnaI polypeptide, the polynucleotide comprising a sequence set out in SEQ ID NO: 1. Such a polynucleotide encodes a full length DnaI gene, or a variant thereof. It is contemplated that this full-length gene is essential to the growth and/or survival of an organism which possesses it, such as *S. aureus*.

As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing a fragment of a full-length DnaI polypeptide, particularly a *S. aureus* DnaI polypeptide or a variant thereof. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

A polynucleotide of the invention is obtained using *S. aureus* cells as starting material, the nucleotide sequence information disclosed in SEQ ID NO: 1, and standard cloning and screening methods, such as those for cloning and sequencing chromosomal DNA fragments from bacteria. For example, to obtain a polynucleotide sequence of the invention, such as the polynucleotide sequence disclosed as in SEQ ID NO: 1, a library of clones of chromosomal DNA of *S. aureus* in *E. coli* or another suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can be distinguished using stringent hybridization conditions. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is of a overnight incubation of a hybridization support (e.g., a nylon or nitrocellulose membrane at 42°C in a solution comprising: 1 X 10^6 cpm/ml labeled probe, 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known to those skilled in the art and are exemplified in Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly

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Chapter 11 therein. Solution hybridization may also be used with the polynucleotide sequences provided by the invention. By sequencing the individual clones thus identified by hybridization, it is possible to confirm the identity of the clone.

Alternatively, an amplification process can be utilized to isolate the polynucleotide. In this approach, the sequence disclosed as SEQ ID NO: 1 is targeted by two oligonucleotides, one identical to a sequence on the coding DNA strand at or upstream of the ATG initiation codon and the other which anneals to the opposite strand at or downstream of the stop codon. Priming from these oligonucleotides in a polymerase chain reaction yields a full length gene coding sequence. Such suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook, MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989).

In a further aspect, the present invention provides for an isolated polynucleotide comprising or consisting of: (a) a polynucleotide sequence which has at least 60% identity, preferably at least 70% identity, more preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95%, most preferably at least 97-99% or exact identity, to that of SEQ ID NO: 1 over the entire length of SEQ ID NO: 1; (b) a polynucleotide sequence encoding a polypeptide which has at least 50% identity, preferably at least 60% identity, more preferably at least 70% identity, more preferably at least 80% identity, more preferably at least 90%, yet more preferably at least 95%, most preferably at least 97-99% or exact identity to SEQ ID NO:2 over the entire length of SEQ ID NO:2; or the complement of a sequence of (a) or (b) above.

The invention provides a polynucleotide sequence identical over its entire length to the coding sequence of SEQ ID NO: 1. Also provided by the invention is a coding sequence for a

mature polypeptide or a fragment thereof (Including, for example, a fragment encoding a polypeptide of SEQ ID NO: 16), by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro-, or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize or destabilize mRNAs, introns, and polyadenylation signals. The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz et al., Proc. Natl. Acad. Sci. 86: 821-824 (1989), or an HA peptide tag (Wilson et al., Cell 37: 767 (1984), both of which may be useful in purifying polypeptide sequences fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

It is most preferred that a polynucleotide of the invention is derived from *Staphylococcus aureus*, however, it may also be obtained from other organisms of the same taxonomic genus. A polynucleotide of the invention may also be obtained, for example, from organisms of the same taxonomic family or order.

Further preferred embodiments are polynucleotides encoding *S. aureus* dnaI variants that have the amino acid sequence of *S. aureus* DnaI polypeptide of SEQ ID NO: 2 in which several,

a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these polynucleotides are those encoding silent nucleotide alterations that do not alter the coding sequence or activities of *S. aureus* DnaI polypeptides they encode.

Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of SEQ ID NO: 1.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to *S. aureus* dnaI polynucleotide sequences, such as those polynucleotides in Fig. 1.

The polynucleotides of the invention are useful as hybridization probes for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding genes that have a high degree of sequence identity to the dnaI gene. Such probes generally will comprise at least 15 to about 100 residues or base pairs, although such probes will preferably have about 20 to 50 nucleotide residues or base pairs. Particularly preferred probes are about 20 to about 30 nucleotide residues or base pairs in length.

A coding region of a related dnaI gene from a bacterial species other than *S. aureus* may be isolated by screening a library using a DNA sequence provided in SEQ ID NO: 1 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine to which member(s) of the library the probe hybridizes.

There are several methods available and well known to those skilled in the art to obtain full-length DNAs, or extend short DNAs, for example those based on the method of Rapid

Amplification of cDNA ends (RACE) (see, for example, Frohman, et al., PNAS USA 85: 8998-9002, 1988). Recent modifications of the technique, exemplified by the MARATHON TM technology (Clontech Laboratories Inc.) for example, have significantly simplified the search for longer cDNAs. In the MARATHON TM technology, cDNAs are prepared from mRNA extracted from a chosen cell and an 'adaptor' sequence is ligated onto each end. Nucleic acid amplification by PCR is then carried out to amplify the "missing" 5' end of the DNA using a combination of gene specific and adaptor specific oligonucleotide primers. The PCR reaction is then repeated using "nested" primers, that is, primers designed to anneal within the amplified product (typically an adaptor-specific primer that anneals further 3' in the adaptor sequence and a gene-specific primer that anneals further 5' in the selected gene sequence). The products of this reaction can then be analyzed by DNA sequencing and a full-length DNA constructed either by joining the product directly to the existing DNA to give a complete sequence, or by carrying out a separate full-length PCR using the new sequence information for the design of the 5' primer.

The polynucleotides and polypeptides of the invention may be employed, for example, as research reagents and materials for discovery of treatments of and diagnostics for diseases, particularly human diseases, as further discussed herein relating to polynucleotide assays.

The polynucleotides of the invention that are oligonucleotides derived from a sequence of SEQ ID NO:1 are useful for the design of PCR primers in reactions to determine whether or not the polynucleotides identified herein in whole or in part are transcribed in bacteria in infected tissue. That is, the polynucleotides of the invention are useful for diagnosis of infection with a bacterial strain carrying those sequences. It is recognized that such sequences also have utility in diagnosis of the stage of infection and type of infection the pathogen has attained.

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The invention also provides polynucleotides that encode a polypeptide that is the mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the mature polypeptide. Such sequences may play a role in processing of a protein from precursor to a mature form, may allow protein transport, may lengthen or shorten protein half-life or may facilitate manipulation of a protein for assay or production, among other things. As generally is the case in vivo, the additional amino acids may be processed away from the mature protein by cellular enzymes.

A precursor protein, having a mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When prosequences are removed such inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called proproteins.

A polynucleotide of the invention thus may encode a mature protein, a mature protein plus a leader sequence (which may be referred to as a preprotein), a precursor of a mature protein having one or more prosequences that are not the leader sequences of a preprotein, or a preproprotein, which is a precursor to a proprotein, having a leader sequence and one or more prosequences, which generally are removed during processing steps that produce active and mature forms of the polypeptide.

In addition to the standard A, G, C, T/U representations for nucleotides, the term "N" may also be used in describing certain polynucleotides of the invention. "N" means that any of the four DNA or RNA nucleotides may appear at such a designated position in the DNA or RNA sequence, except it is preferred that N is not a nucleotide that when taken in combination with adjacent nucleotide positions, read in the correct reading frame, would have the effect of generating a premature termination codon in such reading frame.

For each and every polynucleotide of the invention there is also provided a polynucleotide complementary to it.

Vectors, Host Cells, and Expression Systems

The invention also relates to vectors that comprise a polynucleotide or polynucleotides of the invention, host cells that are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the invention

Recombinant DnaI polypeptides of the present invention may be prepared by processes well known to those skilled in the art from genetically engineered host cells comprising expression systems. Accordingly, in a further aspect, the present invention relates to expression systems that comprise a dnaI polynucleotide or polynucleotides of the present invention, to host cells which are genetically engineered with such expression systems, and to the production of polypeptides of the invention by recombinant techniques.

For recombinant production of DnaI polypeptides of the invention, host cells can be genetically engineered to incorporate expression systems or portions thereof or polynucleotides of the invention. Representative examples of appropriate hosts include bacterial cells (Gram positive and Gram negative), fungal cells, insect cells, animal cells and plant cells. Polynucleotides are introduced to bacteria by standard chemical treatment protocols, such as the induction of competence to take up DNA by treatment with calcium chloride (Sambrook et al., supra). Introduction of polynucleotides into fungal (e.g., yeast) host cells is effected, if desired, by standard chemical methods, such as lithium acetate - mediated transformation.

A great variety of expression systems are useful to produce DnaI polypeptides of the invention. Such vectors include among others, chromosomal-, episomal- and virus-derived vectors. For example, vectors derived from bacterial plasmids, from bacteriophages, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses, and from vectors derived from combinations thereof, are useful in the invention.

DnaI polypeptides of the invention are recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid or urea extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, and lectin chromatography. Well known techniques for refolding may be employed to regenerate an active conformation when the DnaI polypeptide is denatured during isolation and/or purification.

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Diagnostic, Prognostic, Serotyping, and Mutation Assays

This invention is also related to the use of dnaI polynucleotides and polypeptides of the invention for use as diagnostic reagents. Detection of *S. aureus* dnaI polynucleotides and/or polypeptides in a eukaryote, particularly a mammal, and especially a human, will provide a diagnostic method for diagnosis of disease, staging of disease or response of an infectious organism to drugs. Eukaryotes, particularly mammals, and especially humans, particularly those infected or suspected to be infected with an organism comprising the *S. aureus* dnaI gene or protein, may be detected at the nucleic acid or amino acid level by a variety of well known techniques as well as by methods provided herein.

Polypeptides and polynucleotides for prognosis, diagnosis or other analysis may be obtained from a putatively infected and/or infected individual's bodily materials. Polynucleotides

from any of these sources, particularly DNA or RNA, may be used directly for detection or may be amplified enzymatically by using PCR or any other amplification technique prior to analysis. RNA, particularly mRNA, cDNA and genomic DNA may also be used in the same ways. Using amplification, characterization of the species and strain of infectious or resident organism present in an individual, may be made by an analysis of the genotype of a selected polynucleotide of the organism. Deletions and insertions can be detected by a change in size of the amplified product in comparison to a genotype of a reference sequence selected from a related organism, preferably a different species of the same genus or a different strain of the same species. Point mutations can be identified by hybridizing amplified DNA to labeled dnaI polynucleotide sequences. Perfectly or significantly matched sequences can be distinguished from imperfectly or more significantly mismatched duplexes by DNase or RNase digestion, for DNA or RNA respectively, or by detecting differences in melting temperatures or renaturation kinetics. Polynucleotide sequence differences may also be detected by alterations in the electrophoretic mobility of polynucleotide fragments in gels as compared to a reference sequence. This may be carried out with or without denaturing agents. Polynucleotide differences may also be detected by direct DNA or RNA sequencing. See, for example, Myers et al, (1985) Science **230,** 1242. Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase, V1 and S1 protection assay or a chemical cleavage method. See, for example, Cotton et al., (1985) Proc. Natl. Acad. Sci., USA **85,** 4397-4401.

In another embodiment, an array of oligonucleotide probes comprising dnaI nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of, for example, genetic mutations, serotype, taxonomic classification or identification. Array technology methods are well known and have general applicability and can be used to address a variety of

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questions in molecular genetics including gene expression, genetic linkage, and genetic variability (see, for example, Chee et al., (1996) Science 274, 610).

Thus in another aspect, the present invention relates to a diagnostic kit which comprises: (a) a polynucleotide of the present invention, preferably the nucleotide sequence of SEQ ID NO: 1, or a fragment thereof ; (b) a nucleotide sequence complementary to that of (a); (c) a polypeptide of the present invention, preferably the polypeptide of SEQ ID NO:2 or a fragment thereof; or (d) an antibody to a polypeptide of the present invention, preferably to the polypeptide of SEQ ID NO:2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component. Such a kit will be of use in diagnosing a disease or susceptibility to a disease, among others.

This invention also relates to the use of dnaI polynucleotides of the present invention as diagnostic reagents. Detection of a mutated form of a polynucleotide of the invention, preferably, SEQ ID NO: 1, which is associated with a disease or pathogenicity will provide a diagnostic tool that can add to, or define, a diagnosis of a disease, a prognosis of a course of disease, a determination of a stage of disease, or a susceptibility to a disease, which results from under-expression, over-expression or altered expression of the polynucleotide. Organisms, particularly infectious organisms, carrying mutations in such polynucleotide may be detected at the polynucleotide level by a variety of techniques, such as those described elsewhere herein.

The dnaI nucleotide sequences of the present invention are also valuable for organism chromosome identification. The sequence is specifically targeted to, and can hybridize with, a particular location on an organism's chromosome, particularly to a *S. aureus* chromosome. The mapping of relevant sequences to chromosomes according to the present invention may be an

important step in correlating those sequences with pathogenic potential and/or an ecological niche of an organism and/or drug resistance of an organism, as well as the essentiality of the gene to the organism. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data may be found on-line in a sequence database. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through known genetic methods, for example, through linkage analysis (coinheritance of physically adjacent genes) or mating studies, such as by conjugation.

The differences in a polynucleotide and/or polypeptide sequence between organisms possessing a first phenotype and organisms possessing a different, second different phenotype can also be determined. If a mutation is observed in some or all organisms possessing the first phenotype but not in any organisms possessing the second phenotype, then the mutation is likely to be the causative agent of the first phenotype.

Polypeptides and polynucleotides for prognosis, diagnosis or other analysis may be obtained from a putatively infected and/or infected individual's bodily materials. Particularly DNA or polynucleotides, from any of these sources may be used directly for detection or may be amplified enzymatically using PCR or other amplification technique with oligonucleotide amplification primers derived from the polynucleotide sequence of *S. aureus* dna 1. RNA, particularly mRNA, or RNA reverse transcribed to cDNA, is also useful for diagnostics. Following amplification of a *S aureus* dnal - related polynucleotide from a sample, characterization of the species and strain of infecting or resident organism is made by an analysis of the amplified polynucleotide relative to one or more reference polynucleotides or sequences relative to a standard from a related organism (i.e. a known strain of *S. aureus*).

Point mutations can be identified by hybridizing amplified DNA to known dnaI polynucleotide sequences and by detecting differences in melting temperatures or renaturation kinetics. Perfectly or significantly matched sequences can be distinguished from imperfectly or more significantly mismatched duplexes by RNase protection or S1 nuclease mapping. (See, for example, Cotton et al., (1988) Proc. Natl. Acad. Sci. USA 85:4397-4401). Polynucleotide sequence differences may also be detected by alterations in the electrophoretic mobility of polynucleotide fragments in gels as compared to a reference sequence. This may be carried out with or without denaturing agents. Polynucleotide differences may also be detected by direct DNA or RNA sequencing. See, for example, Myers et al, (1985) Science 230, 1242. Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase, V1 and S1 protection assay or a chemical cleavage method.(Cotton et al., 1988 Supra).

In another embodiment, an array of oligonucleotide probes comprising dnaI nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of, for example, genetic mutations, serotype, taxonomic classification or identification. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability (see, for example, Chee et al., (1996) Science 274, 610).

In another aspect, the present invention relates to a diagnostic kit which comprises: (a) a polynucleotide of the present invention, preferably the nucleotide sequence of SEQ ID NO: 1, or a fragment thereof; (b) a nucleotide sequence complementary to that of (a); (c) a polypeptide of the present invention, preferably the polypeptide of SEQ ID NO:2 or a fragment thereof; or (d) an antibody to a polypeptide of the present invention, preferably to the polypeptide of SEQ ID

NO:2. Such a kit will be of use in diagnosing a disease or susceptibility to a disease, among other uses.

The invention further provides a process for diagnosing bacterial infections such as those caused by *S. aureus*, the process comprising determining from a sample derived from an individual, such as a bodily material, an increased level of expression of a polynucleotide having a sequence disclosed in SEQ ID NO: 1 relative to a sample taken from a non-diseased individual. Increased or decreased expression of a dnaI polynucleotide can be measured using any one of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods, and spectrometry.

In addition, a diagnostic assay in accordance with the invention for detecting over-expression of DnaI polypeptide compared to normal control tissue samples may be used to detect the presence of an infection, for example. Assay techniques that can be used to determine levels of a *S. aureus* DnaI polypeptide, in a sample derived from a host, such as a bodily material, are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis, antibody sandwich assays, antibody detection and ELISA assays.

Gridding and Polynucleotide Subtraction of *S. aureus* Genomic Sequences

The dnaI polynucleotides of the invention may be used as components of polynucleotide arrays, preferably high density arrays or grids. These high density arrays are particularly useful for diagnostic and prognostic purposes. For example, a set of spots each comprising a different gene, and further comprising a polynucleotide or polynucleotides of the invention, may be used for probing, such as hybridization or nucleic acid amplification, using a probe obtained or

derived from a bodily sample, to determine the presence a particular polynucleotide sequence or related sequence in an individual.

Antibodies Specific for *S. aureus* Peptides or Polypeptides

The DnaI polypeptides and polynucleotides of the invention or variants thereof, or cells expressing them are useful as immunogens to produce antibodies immunospecific for such polypeptides or polynucleotides, respectively.

In certain preferred embodiments of the invention there are provided antibodies against *S. aureus* DnaI polypeptides or polynucleotides encoding them. Antibodies against DnaI-polypeptide or dnaI-polynucleotide are useful for treatment of infections, particularly bacterial infections.

Antibodies generated against the polypeptides or polynucleotides of the invention are obtained by administering the polypeptides and/or polynucleotides of the invention or epitope-bearing fragments of either or both, analogues of either or both, or cells expressing either or both, to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique known in the art that provides antibodies produced by continuous cell line cultures is useful. Examples include various techniques, such as those in Kohler, G. and Milstein, C., Nature 256: 495-497 (1975); Kozbor et al., Immunology Today 4: 72 (1983); and Cole et al., pg. 77-96 in MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc. (1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can be adapted to produce single chain antibodies to polypeptides or polynucleotides of this invention. Also, transgenic mice, or other mammals, are useful to express humanized antibodies immunospecific to the polypeptides or polynucleotides of the invention.

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When antibodies are administered therapeutically, the antibody or variant thereof is preferably modified to make it less immunogenic in the individual. For example, if the individual is human the antibody is most preferably "humanized," where the complimentarity determining region or regions of the hybridoma-derived antibody has been transplanted into a human monoclonal antibody, for example as described in Jones et al. (1986), Nature 321, 522-525 or Tempest et al., (1991) Biotechnology 9, 266-273.

Alternatively, phage display technology is useful to select antibody genes with binding activities towards a DnaI polypeptide of the invention. In one approach, antibody fragments specific for *S. aureus* DnaI are selected from an immune library of antibody genes expressed as fusions with coat protein of filamentous phage. Alternatively, naive libraries are screened by phage display techniques to identify genes encoding antibodies specific for DnaI or from naive libraries (McCafferty, et al., (1990), Nature 348, 552-554; Marks, et al., (1992) Biotechnology 10, 779-783; a recent reference is de Haard *et al.* (1999) J Biol Chem **274**: 18218-18230). The ability to recover, for various targets, antibodies with subnanomolar affinities obviates the need for immunization. The affinity of these antibodies can also be improved by, for example, chain shuffling (Clackson et al., (1991) Nature 352: 628).

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptides or polynucleotides of the invention, for example to purify the polypeptides or polynucleotides by immunoaffinity chromatography.

A variant polypeptide or polynucleotide of the invention, such as an antigenically or immunologically equivalent derivative or a fusion protein of the polypeptide is also useful as an antigen to immunize a mouse or other animal such as a rat or chicken. A fused protein provides stability to the polypeptide acting as a carrier, or acts as an adjuvant or both. Alternatively, the

antigen is associated, for example by conjugation, with an immunogenic carrier protein, such as bovine serum albumin, keyhole limpet haemocyanin or tetanus toxoid. Alternatively, when antibodies are to be administered therapeutically, alternatively a multiple antigenic polypeptide comprising multiple copies of the polypeptide, or an antigenically or immunologically equivalent polypeptide thereof may be sufficiently antigenic to improve immunogenicity so as to obviate the use of a carrier.

In accordance with an aspect of the invention, there is provided the use of a dnaI polynucleotide of the invention for therapeutic or prophylactic purposes, in particular genetic immunization. The use of a dnaI polynucleotide of the invention in genetic immunization preferably employs a suitable delivery method such as direct injection of plasmid DNA into muscles (Wolff et al., Hum Mol Genet (1992) 1: 363, Manthorpe et al., Hum. Gene Ther. (1983) 4: 419), delivery of DNA complexed with specific protein carriers (Wu et al., J. Biol. Chem. (1989) 264: 16985), coprecipitation of DNA with calcium phosphate (Benvenisty & Reshef, PNAS USA, (1986) 83: 9551), encapsulation of DNA in various forms of liposomes (Kaneda et al., Science (1989) 243: 375), particle bombardment (Tang et al., Nature (1992) 356:152, Eisenbraun et al., DNA Cell Biol (1993) 12: 791) or in vivo infection using cloned retroviral vectors (Seeger et al., PNAS USA (1984) 81: 5849).

Antagonists and Agonists: Assays and Molecules

The invention is based in part on the discovery that DnaI is a target for the bacteria phage 77ORF104 inhibitory factor. Applicants have recognized the utility of the interaction in the development of antibacterial agents. Specifically, the inventors have recognized that 1) DnaI is a critical target for bacterial inhibition; 2) 77ORF104 or derivatives or functional mimetics thereof are useful for inhibiting bacterial growth; and 3) the interaction between dnaI and of *S. aureus*

and 77ORF104 may be used as a target for the screening and rational design of drugs or antibacterial agents. In addition to methods of directly inhibiting DnaI activity, methods of inhibiting DnaI expression are also attractive for antibacterial activity.

In several embodiments of the invention, there are provided methods for identifying compounds which bind to or otherwise interact with and inhibit or activate an activity or expression of a polypeptide and/or polynucleotide of the invention comprising: contacting a polypeptide and/or polynucleotide of the invention with a compound to be screened under conditions to permit binding to or other interaction between the compound and the polypeptide and/or polynucleotide to assess the binding to or other interaction with the compound, such binding or interaction preferably being associated with a second component capable of providing a detectable signal in response to the binding or interaction of the polypeptide and/or polynucleotide with the compound; and determining whether the compound binds to or otherwise interacts with and activates or inhibits an activity or expression of the polypeptide and/or polynucleotide by detecting the presence or absence of a signal generated from the binding or interaction of the compound with the polypeptide and/or polynucleotide.

Potential antagonists include, among others, small organic molecules, peptides, polypeptides and antibodies that bind to a polynucleotide and/or polypeptide of the invention and thereby inhibit or extinguish its activity or expression. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a binding molecule, without inducing dnaI-induced activities, thereby preventing the action or expression of *S. aureus* DnaI polypeptides and/or polynucleotides by excluding *S. aureus* DnaI polypeptides and/or polynucleotides from binding.

Potential antagonists also include a small molecule that binds to and occupies the binding site of the polypeptide thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented. Examples of small molecules include but are not limited to small organic molecules, peptides or peptide-like molecules. Other potential antagonists include antisense molecules (see Okano, (1991) J. Neurochem. **56,** 560; see also OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION, CRC Press, Boca Raton, FL (1988), for a description of these molecules). Preferred potential antagonists include compounds related to and variants of 77ORF104 and of DnaI. Other examples of potential polypeptide antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligands, substrates, receptors, enzymes, etc., as the case may be, of the polypeptide, e.g., a fragment of the ligands, substrates, receptors, enzymes, etc.; or small molecules which bind to the polypeptide of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented.

Compounds may be identified from a variety of sources, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See, e.g., Coligan et al., Current Protocols in Immunology 1(2): Chapter 5 (1991). Peptide modulators can also be selected by screening large random libraries of all possible peptides of a certain length.

Compounds could also be derived from the polypeptide sequence of 77ORF104 itself. Peptide fragments representing small overlapping fragments or peptides spanning the entire amino acid sequence of the protein can be used to perform extensive screens. Fragments of 77ORF104 can be produced by proteolytic digestion of the full-length protein as described above. Alternatively, suitable 77ORF104-derived peptide or polypeptide fragments

representative of the complete sequence of the protein can be chemically synthesized. For instance, in the multi-pin approach, peptides are simultaneously synthesized by the assembly of small quantities of peptides on plastic pins derivatized with an ester linker based on glycolate and 4-(hydroxymethyl) benzoate (Maeji *et al.* (1991) Pept Res, **4**:142-6).

Certain of the polypeptides of the invention are biomimetics, functional mimetics of the natural *S. aureus* DnaI polypeptide. These functional mimetics are useful for, among other things, antagonizing the activity of *S. aureus* DnaI polypeptide or as an antigen or immunogen in a manner described above. Functional mimetics of the polypeptides of the invention include but are not limited to truncated polypeptides. For example, preferred functional mimetics include a polypeptide comprising the polypeptide sequence set forth in SEQ ID NO: 2 lacking 20, 30, 40, 50, 60, 70 or 80 amino- or carboxy-terminal amino acid residues, including fusion proteins comprising one or more of these truncated sequences. Polynucleotides encoding each of these functional mimetics may be used as expression cassettes to express each mimetic polypeptide. It is preferred that these cassettes comprise 5' and 3' restriction sites to allow for a convenient means to ligate the cassettes together when desired. It is further preferred that these cassettes comprise gene expression signals known in the art or described elsewhere herein.

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Screening Assays According to the Invention

It is desirable to devise screening methods to identify compounds which stimulate or which inhibit the function of the DnaI polypeptide or polynucleotide of the invention. Accordingly, the present invention provides for a method of screening compounds to identify those that modulate the function of a polypeptide or polynucleotide of the invention. In general, antagonists may be employed for therapeutic and prophylactic purposes. It is contemplated that

an agonist of DnaI may be useful, for example, to enhance the growth rate of bacteria in a sample being cultured for diagnostic or other purposes.

Screening methods generally fall into two broad categories: those that assay binding of candidate compounds; and those that assay a functional aspect of the target.

a) Binding Assays

There are a number of methods of examining binding of a candidate compound to a protein target such as DnaI. Screening methods that measure the binding of a candidate compound to the DnaI polypeptide or polynucleotide, or to cells or supports bearing the polypeptide or a fusion protein comprising the polypeptide, by means of a label directly or indirectly associated with the candidate compound, are useful in the invention.

The screening method may involve competition for binding of a labeled competitor such as 77ORF104 or a fragment that is competent to bind DnaI.

i) Phage display

Phage display is a powerful assay to measure protein:protein interaction. In this scheme, proteins or peptides are expressed as fusions with coat proteins or tail proteins of filamentous bacteriophage. A comprehensive monograph on this subject is *Phage Display of Peptides and Proteins. A Laboratory Manual* edited by Kay *et al.* (1996) Academic Press. For phages in the Ff family that include M13 and fd, gene III protein and gene VIII protein are the most commonly-used partners for fusion with foreign protein or peptides. Phagemids are vectors containing origins of replication both for plasmids and for bacteriophage. Phagemids encoding fusions to the gene III or gene VIII can be rescued from their bacterial hosts with helper phage, resulting in the display of the foreign sequences on the coat or at the tip of the recombinant phage.

In the simplest assay, purified recombinant DnaI protein, or a fragment of DnaI, for example comprising the amino acid sequence of SEQ ID NO: 16, could be immobilized in the wells of a microtitre plate and incubated with phages displaying 77ORF104 in fusion with the gene III protein. Washing steps are performed to remove unbound phages and bound phages are detected with monoclonal antibodies directed against phage coat protein (gene VIII protein). Color development by means of an enzyme-linked secondary antibody allows quantitative detection of bound fusion protein. Screening for inhibitors is performed by the incubation of the compound with the immobilized target before the addition of phages. The presence of an inhibitor will specifically reduce the signal in a dose-dependent manner relative to controls without inhibitor.

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ii) Surface plasmon resonance

Another powerful assay to screen for inhibitors of a for protein: protein interaction is surface plasmon resonance. Surface plasmon resonance is a quantitative method that measures binding between two (or more) molecules by the change in mass near the sensor surface caused by the binding of one protein or other biomolecule from the aqueous phase to a second protein or biomolecule immobilized on the sensor. This change in mass is measured as resonance units versus time after injection or removal of the second protein or biomolecule and is measured using a Biacore Biosensor (Biacore AB). DnaI could be immobilized on a sensor chip (for example, research grade CM5 chip; Biacore AB) using a covalent linkage method (e.g. amine coupling in 10 mM sodium acetate [pH 4.5]). A blank surface is prepared by activating and inactivating a sensor chip without protein immobilization. The binding of 77ORF104 to DnaI, or a fragment of DnaI, for example comprising the amino acid sequence of SEQ ID NO: 16, is measured by injecting purified 77ORF104 over the chip surface. Measurements are performed at

room temperature. Conditions used for the assay (i.e., those permitting binding) are as follows: 25 mM HEPES-KOH (pH 7.6), 150 mM sodium chloride, 15% glycerol, 1 mM dithiothreitol, and 0.001% Tween 20 with a flow rate of 10 ul/min. Preincubation of the sensor chip with candidate inhibitors will predictably decrease the interaction between 77ORF104 and DnaI. A decrease in 77ORF104 binding is indicative of competitive binding by the candidate compound.

iii) Fluorescence Resonance Energy Transfer (FRET)

Another method of measuring inhibition of binding of two proteins uses fluorescence resonance energy transfer (FRET; de Angelis, 1999, Physiological Genomics). FRET is a quantum mechanical phenomenon that occurs between a fluorescence donor (D) and a fluorescence acceptor (A) in close proximity (usually < 100 A of separation.) if the emission spectrum of D overlaps with the excitation spectrum of A. Variants of the green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* are fused to a polypeptide or protein and serve as D-A pairs in a FRET scheme to measure protein-protein interaction. Cyan (CFP: D) and yellow (YFP: A) fluorescence proteins are linked with DnaI polypeptide, or a fragment of DnaI, for example comprising the amino acid sequence of SEQ ID NO: 16, and 77ORF104 protein respectively. Under optimal proximity, interaction between DnaI, or a fragment of DnaI, for example comprising the amino acid sequence of SEQ ID NO: 16 and 77ORF104 causes a decrease in intensity of CFP concomitant with an increase in YFP fluorescence.

The addition of a candidate modulator to the mixture of appropriately labeled DnaI and 77ORF104 protein, will result in an inhibition of energy transfer evidenced by, for example, a decease in YFP fluorescence at a given concentration of 77ORF104 relative to a sample without the candidate inhibitor.

iv) Fluorescence polarization

In addition to the surface plasmon resonance and FRET methods, fluorescence polarization measurement is useful to quantitate protein-protein binding. The fluorescence polarization value for a fluorescently-tagged molecule depends on the rotational correlation time or tumbling rate. Protein complexes, such as those formed by *S. aureus* DnaI polypeptide, or a fragment of DnaI, for example comprising the amino acid sequence of SEQ ID NO: 16 associating with a fluorescently labeled polypeptide (e.g., 77ORF104 or a binding fragment thereof), have higher polarization values than a fluorescently labeled monomeric protein. Inclusion of a candidate inhibitor of the DnaI interaction results in a decrease in fluorescence polarization relative to a mixture without the candidate inhibitor if the candidate inhibitor disrupts or inhibits the interaction of DnaI with its polypeptide binding partner. It is preferred that this method be used to characterize small molecules that disrupt the formation of polypeptide or protein complexes.

v) Scintillation Proximity Assay

A scintillation proximity assay may be used to characterize the interaction between a *S. aureus* DnaI polypeptide, or a fragment of DnaI polypeptide, for example comprising the amino acid sequence of SEQ ID NO: 16 and another polypeptide. For the assay, *S. aureus* DnaI polypeptide can be covalently coupled to beads. Addition of radio-labeled 77ORF104 results in binding where the radioactive source molecule is in close proximity to the scintillation fluid. Thus, signal is emitted upon 77ORF104 polypeptide binding, and compounds that prevent association between *S. aureus* DnaI polypeptide and 77ORF104 diminish the scintillation signal.

vi) Bio Sensor Assay

ICS biosensors have been described by AMBRI (Australian Membrane Biotechnology Research Institute; http//www.ambri.com.au/). In this technology, the self-association of macromolecules such as DnaI, or a fragment of DnaI, for example comprising the amino acid

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sequence of SEQ ID NO: 16, and bacteriophage 77 ORF 104, is coupled to the closing of gramacidin-facilitated ion channels in suspended membrane bilayers and hence to a measurable change in the admittance (similar to impedence) of the biosensor. This approach is linear over six order of magnitude of admittance change and is ideally suited for large scale, high through-put screening of small molecule combinatorial libraries.

It is important to note that in assays of protein-protein interaction, it is possible that a modulator of the interaction need not necessarily interact directly with the domain(s) of the proteins that physically interact. It is also possible that a modulator will interact at a location removed from the site of protein-protein interaction and cause, for example, a conformational change in the DnaI polypeptide. Modulators (inhibitors or agonists) that act in this manner are of interest since the change they induce may modify the activity of the DnaI polypeptide.

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b. Assays of DnaI Functional Activity.

i) Assay for DNA replication, ^{3}H-thymidine incorporation

To measure the effect of 77ORF104 expression on *S. aureus* DNA replication, the level of radiolabeled thymidine incorporation into DNA is measured in the presence or in the absence of sodium arsenite (5uM). Samples (0.5 ml) are withdrawn from the cultures at appropriate time intervals and mixed to 4.5 ul of labeling solution (0.2 uCi/ml of ^{3}H-thymidine (73 Ci/mmol, NEN Life Science Products, Inc) and 70 pmol of cold thymidine). After 15 min of reaction, incorporation is stopped by adding solution containing 0.2% NaN_3 and 1 mM cold thymidine. Samples are precipitated with 10% w/v trichloroacetic acid and filtered through glass fiber filters (GF-C, Whatman). The results are expressed as ^{3}H-thymidine counts incorporated normalized to OD culture.

The assay is performed in the presence of varying concentrations of candidate inhibitors in place of 77 ORF104 to screen for inhibitors. At least a 10-fold reduction in ^{3}H-thymidine incorporation in the presence of 77 ORF104 or other inhibitor indicates a reduction in DnaI activity.

ii) Plasmid replication

The plasmid pC194 replicates in *S. aureus* by rolling circle mechanism. The single stranded origin, sso of the pC194 is involved in the synthesis of the lagging DNA strand. The plasmid pADG6406 is a derivative of pC194 lacking sso. The absence of sso leads the accumulation of plasmid single-stranded DNA. The single-stranded (ss) initiation site, ssiA, is located on the lagging strand of pAM 1 and is a site for primosome assembly. SsiA was inserted into plasmid pADG6404. *S aureus* harboring plasmids are grown to mid-log phase and their total DNA is extracted and analyzed by Southern hybridization, using ^{32}P-labeled plasmid DNA as probe. The presence of pADG6406 with ssiA is associated to a decrease in the ratio of ss to double stranded (ds) DNA compared to that of the plasmid without ssiA. This system is used to measure the effect of 77ORF104 or a candidate inhibitor polypeptideexpression on ss DNA synthesis. In an assay, a plasmid containing 77ORF104 or a candidate inhibitor polypeptide coding sequence under an arsenite inducible promotor is introduced into a *S aureus* strain harboring pADG6406. The ratio of ss to ds DNA of pADG6406 is measured in the presence or in the absence of sodium arsenite (5 uM). An increase in the ratio of ss to ds DNA (10% or more) indicates an effect of the candidate modulator. In another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, etc. for a polypeptide and/or polynucleotide of the present invention; or compounds which decrease or enhance the production of such polypeptides and/or polynucleotides, which

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comprises: (a) a polypeptide and/or a polynucleotide of the present invention; (b) a recombinant cell expressing a polypeptide and/or polynucleotide of the present invention; (c) a cell membrane expressing a polypeptide and/or polynucleotide of the present invention; or (d) antibody to a polypeptide and/or polynucleotide of the present invention; which polypeptide is preferably that of SEQ ID NO: 2, and which polynucleotide is preferably that of SEQ ID NO: 1.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

It will be readily appreciated by the skilled artisan that a polypeptide and/or polynucleotide of the present invention may also be used in a method for the structure-based design of an agonist, antagonist or inhibitor of the polypeptide and/or polynucleotide, by: (a) determining in the first instance the three-dimensional structure of the polypeptide and/or polynucleotide, or complexes thereof; (b) deducing the three-dimensional structure for the likely reactive site(s), binding site(s) or motif(s) of an agonist, antagonist or inhibitor; (c) synthesizing candidate compounds that are predicted to bind to or react with the deduced binding site(s), reactive site(s), and/or motif(s); and (d) testing whether the candidate compounds are indeed agonists, antagonists or inhibitors. It will be further appreciated that this will normally be an iterative process, and this iterative process may be performed using automated and computer-controlled steps.

Each of the polynucleotide sequences provided herein may be used in the discovery and development of antibacterial compounds. The encoded protein, upon expression, can be used as a target for the screening of antibacterial drugs. Additionally, the polynucleotide sequences encoding the amino terminal regions of the encoded protein or Shine-Delgarno or other

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translation facilitating sequences of the respective mRNA can be used to construct antisense sequences to control the expression of the coding sequence of interest.

The invention also provides the use of the polypeptide, polynucleotide, agonist or antagonist of the invention to interfere with the initial physical interaction between a pathogen or pathogens and a eukaryotic, preferably mammalian, host that is responsible for sequelae of infection. In particular, the molecules of the invention may be used: in the prevention of adhesion of bacteria, in particular Gram positive and/or Gram negative bacteria, to eukaryotic, preferably mammalian, extracellular matrix proteins on in-dwelling devices or to extracellular matrix proteins in wounds; to block bacterial adhesion between eukaryotic, preferably mammalian, extracellular matrix proteins and bacterial DnaI proteins that mediate tissue damage and/or; to block the normal progression of pathogenesis in infections initiated other than by the implantation of in-dwelling devices or by other surgical techniques.

In accordance with yet another aspect of the invention, there are provided dnaI antagonists, preferably bacteriostatic or bacteriocidal antagonists.

The antagonists of the invention may be employed, for instance, to prevent, inhibit and/or treat diseases.

Compositions, kits and administration

In a further aspect of the invention there are provided compositions comprising a dnaI polynucleotide and/or a *S. aureus* DnaI polypeptide for administration to a cell or to a multicellular organism.

The present invention provides for pharmaceutical compositions comprising a therapeutically effective amount of a polypeptide and/or polynucleotide, such as the soluble form of a polypeptide and/or polynucleotide of the present invention, antagonist peptide or small

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molecule compound, in combination with a pharmaceutically acceptable carrier or excipient. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.

In therapy or as a prophylactic, the active agent may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic.

Alternatively the composition may be formulated for topical application for example in the form of ointments, creams, lotions, eye ointments, eye drops, ear drops, mouthwash, impregnated dressings and sutures and aerosols, and may contain appropriate conventional additives, including, for example, preservatives, solvents to assist drug penetration, and emollients in ointments and creams. Such topical formulations may also contain compatible conventional carriers, for example cream or ointment bases, and ethanol or oleyl alcohol for lotions. Such carriers may constitute from about 1% to about 98% by weight of the formulation; more usually they will constitute up to about 80% by weight of the formulation. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if a polypeptide or other compounds of the present invention can be formulated in an enteric or an encapsulated formulation, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels, and the like.

For administration to mammals, and particularly humans, it is expected that the daily dosage level of the active agent will be from 0.01 mg/kg to 10 mg/kg, typically around 1 mg/kg.

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The physician in any event will determine the actual dosage which will be most suitable for an individual and will vary with the age, weight and response of the particular individual. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

As used herein, the term "in-dwelling device" refers to surgical implants, prosthetic devices and catheters, i.e., devices that are introduced to the body of an individual and remain in position for an extended time. Such devices include, but are not limited to, artificial joints, heart valves, pacemakers, vascular grafts, vascular catheters, cerebrospinal fluid shunts, urinary catheters, continuous ambulatory peritoneal dialysis (CAPD) catheters.

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The composition of the invention may be administered by injection to achieve a systemic effect against relevant bacteria shortly before insertion of an in-dwelling device. Treatment may be continued after surgery during the in-body time of the device. In addition, the composition could also be used to broaden perioperative cover for any surgical technique to prevent bacterial wound infections, especially *S. aureus* wound infections.

Many orthopedic surgeons consider that humans with prosthetic joints should be considered for antibiotic prophylaxis before dental treatment that could produce a bacteremia. Deep infection is a serious complication sometimes leading to loss of the prosthetic joint and is accompanied by significant morbidity and mortality. It may therefore be possible to extend the use of the active agent as a replacement for prophylactic antibiotics in this situation.

In addition to the therapy described above, the compositions of this invention may be used generally as a wound treatment agent to prevent adhesion of bacteria to matrix proteins exposed in wound tissue and for prophylactic use in dental treatment as an alternative to, or in conjunction with, antibiotic prophylaxis.

Alternatively, the composition of the invention may be used to bathe an indwelling device immediately before insertion. The active agent will preferably be present at a concentration of 1 mg/ml to 10mg/ml for bathing of wounds or indwelling devices.

A vaccine composition is conveniently in injectable form. Conventional adjuvants may be employed to enhance the immune response. A suitable unit dose for vaccination is 0.5-5 microgram/kg of antigen, and such dose is preferably administered 1-3 times and with an interval of 1-3 weeks. With the indicated dose range, no adverse toxicological effects will be observed with the compounds of the invention which would preclude their administration to suitable individuals.

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Sequence Databases, Sequences in a Tangible Medium, and Algorithms

Polynucleotide and polypeptide sequences form a valuable information resource with which to determine their 2- and 3-dimensional structures as well as to identify further sequences of similar homology. These approaches are most easily facilitated by storing the sequence in a computer readable medium and then using the stored data in a known macromolecular structure program or to search a sequence database using well known searching tools, such as GCC.

The polynucleotide and polypeptide sequences of the invention are particularly useful as components in databases useful for search analyses as well as in sequence analysis algorithms. As used in this section entitled "Sequence Databases, Sequences in a Tangible Medium, and Algorithms," and in claims related to this section, the terms "polynucleotide of the invention" and "polynucleotide sequence of the invention" mean any detectable chemical or physical characteristic of a polynucleotide of the invention that is or may be reduced to or stored in a tangible medium, preferably a computer readable form. For example, chromatographic scan data or peak data, photographic data or scan data therefrom, called bases, and mass spectrographic

data. As used in this section entitled Databases and Algorithms and in claims related thereto, the terms "polypeptide of the invention" and "polypeptide sequence of the invention" mean any detectable chemical or physical characteristic of a polypeptide of the invention that is or may be reduced to or stored in a tangible medium, preferably a computer readable form. For example, chromatographic scan data or peak data, photographic data or scan data therefrom, and mass spectrographic data.

The invention provides a computer readable medium having stored thereon polypeptide sequences of the invention and/or polynucleotide sequences of the invention. The computer readable medium can be any composition of matter used to store information or data, including, for example, commercially available floppy disks, tapes, chips, hard drives, compact disks, and video disks.

In a preferred embodiment of the invention there is provided a computer readable medium having stored thereon a member selected from the group consisting of: a polynucleotide comprising the sequence of SEQ ID NO: 1 or SEQ ID NO: 17; a polypeptide comprising the sequence of SEQ ID NO: 2 or SEQ ID NO: 16; a set of polynucleotide sequences wherein at least one of said sequences comprises the sequence of SEQ ID NO: 1 or SEQ ID NO: 17: a set of polypeptide sequences wherein at least one of said sequences comprises the sequence of SEQ ID NO: 2 or SEQ ID NO: 16; a data set representing a polynucleotide sequence comprising the sequence of SEQ ID NO: 1 or SEQ ID NO: 17; a data set representing a polynucleotide sequence encoding a polypeptide sequence comprising the sequence of SEQ ID NO: 2 or SEQ ID NO: 16; a polynucleotide comprising the sequence of SEQ ID NO: 1 or SEQ ID NO: 17; a polypeptide comprising the sequence of SEQ ID NO: 2 or SEQ ID NO: 16; a set of polynucleotide sequences wherein at least one of said sequences comprises the sequence of SEQ ID NO: 1 or SEQ ID NO:

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17; a set of polypeptide sequences wherein at least one of said sequences comprises the sequence of SEQ ID NO: 2 or SEQ ID NO: 16; a data set representing a polynucleotide sequence comprising the sequence of SEQ ID NO: 1 or SEQ ID NO: 17; a data set representing a polynucleotide sequence encoding a polypeptide sequence comprising the sequence of SEQ ID NO: 2 or SEQ ID NO: 16.

All publications and references, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference in their entirety as if each individual publication or reference were specifically and individually indicated to be incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety in the manner described above for publications and references.

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EXAMPLES

Example 1

Identification of the inhibitory ORF 104 from Staphylococcus aureus bacteriophage 77.

The *S. aureus* propagating strain 77 (PS 77) was used as a host to propagate its respective phage 77 (ACTT #27699-B1). The phage was propagated using the agar layer method described by Swanstörm and Adams (Swanström et al. (1951) Proc. Soc. Exptl. Biol. & Med. **78:** 372-375). Phage DNA was prepared from the purified phages as described in Sambrook *et al.* (1989) Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. Blunt-ended sonicated phage DNA fragments were cloned into the pKSII vector (Stratagene). Recombinant clones were sequenced on an ABI 377-36 automated sequencer. To ensure co-linearity of the sequence data and the genome, all regions of the phage

genome were sequenced at least once from both directions on two separate clones. Sequence contigs were assembled using Sequencher 3.1 software (GeneCodes) (Fig. 2). An implementation of the publically available program SEQUIN, available for download at ftp://negi.nlm.nih.gov/sequin/, was used on phage genome sequence to identify all putative ORFs larger than 33 codons (Fig. 3).

The 77ORF104 (SEQ ID NO: 4) was amplified by polymerase chain reaction (PCR) from phage genomic DNA (Fig. 4). For PCR amplification, the sense strand primer starts at the initiation codon and is preceded by a *BamH*I restriction site; the antisense strand starts at the last codon (excluding the stop codon) and is preceded by a *Sal*I restriction site. The PCR product was gel purified and digested with *BamH*I and *Sal*I. The digested PCR product was then ligated into *BamH*I- and *Sal*I-digested pT vector (Fig. 7A), and used to transform *S. aureus* strain RN4220 (Kreiswirth *et al.* (1983) Nature **305**: 709-712). Selection of recombinant clones was performed on Luria-Bertani (LB) agar plates containing 30 μg/ml of kanamycin.

Sodium arsenite ($NaAsO_2$) was used to induce gene expression from the *ars* promoter/operator. The effect of expression of phage 77 ORFs on bacterial cell growth was then evaluated in functional assays on solid medium and in liquid medium. As shown in Fig. 7B, the induction of expression of phage 77ORF104 by plating transformants on semi-solid medium containing 5 μM sodium arsenite results in the inhibition of bacterial growth on solid medium compared to plating in the absence of inducer or plating of control non-inhibitory ORF (phage 77 ORF 19) transformants. As shown in Fig. 7C, the density of the culture, as assessed by colony forming units (CFU), for *S. aureus* clones harboring the 77ORF104 increased over time under non-induced conditions. Similar growth rates were also observed with transformants harboring a non-inhibitory ORF (labeled as 'non killer' on the graphs) under both induced and non-induced

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conditions. At 4 h following induction, the expression of 77ORF104 is cytocidal resulting in a 0.5 log reduction in the number of CFU compared to the number of CFU initially present in the same culture.

Example 2

Identification of a *S. aureus* protein targeted by bacteriophage 77 ORF 104.

To identify *S. aureus* proteins that interact with the bacterial growth inhibitory Staphylococcus bacteriophage 77 ORF 104, a GST-fusion of ORF 104 was generated and the recombinant protein purified and utilized to make a GST/ORF104 affinity column. Cellular extracts prepared from *S. aureus* cells were incubated with the affinity matrix, washed with increasing salt concentrations and different detergents, and the protein elution profile of the washes assessed by SDS-polyacrylamide gel electrophoresis. A protein of molecular mass ~ 40 kDa was specifically eluted from the affinity matrix. Eluted proteins were further characterized to determine the identity of the interacting protein and to validate the interaction of the protein with 77ORF104 as described in detail below.

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A. Generation of GST/ORF 104 recombinant protein.

Bacteriophage 77ORF104 was sub-cloned into pGEX 4T-1 (Pharmacia), an expression vector containing the GST moiety. The gene encoding ORF104 was obtained by digestion of pT/ORF104 (Fig. 7A) with *BamHI* and *SalI*. The DNA fragment containing ORF104 was gel purified by QiaQuick spin column (Qiagen) and ligated into pGEX 4T-1 (which had been previously digested with *BamHI* and *SalI*) to generate pGEX 4T/ORF104. Recombinant expression vectors were identified by restriction enzyme analysis of plasmid minipreps, large-scale DNA preparations were performed with Qiagen columns, and the resulting plasmid was

sequenced. Test expressions in *E. coli* DH5 cells containing the expression plasmids were performed to identify optimal protein expression conditions. *E. coli* DH5 cells containing the pGEX 4T/ORF104 were grown in Luria-Bertani Broth at 37°C to an OD_{600} of 0.4 to 0.6 and induced with 1 mM IPTG at 30°C for 4 hrs.

B. Fusion protein purification.

Cells containing GST/ORF104 fusion protein were suspended in 20 ml lysis buffer/liter of cell culture with GST lysis buffer (20 mM Hepes pH 7.2, 500 mM NaCl, 10 % glycerol, 1 mM DTT, 1mM EDTA, 1mM benzamidine, and 1 PMSF) and lysed by French Pressure cell followed by three bursts of twenty seconds with an ultra-sonicator at 4°C. Triton X-100 was added to the lysate to a final concentration of 0.1% and mixed for 30 minutes at 4°C. The lysate was centrifuged at 4°C for 30 minutes at 10 000 rpm in a Sorval SS34 rotor. The supernatant was applied to a 4 ml glutathione sepharose column pre-equilibrated with lysis buffer and allowed to flow by gravity. The column was washed with 10 column volumes of lysis buffer and eluted in 1.5 ml fractions with GST elution buffer (20 mM Hepes pH 8.0, 500 mM NaCl, 10 % glycerol, 1 mM DTT, 0.1mM EDTA, and 25 mM reduced glutathione). The fractions were analyzed by 12.5% SDS-PAGE (Laemmli) and visualized by staining with Coomassie Brilliant Blue R250 stain to assess the amount of eluted GST/ORF104 protein.

GST/ORF104 (12 mg) was dialyzed overnight against 20 mM Hepes pH 7.5, 150 mM NaCl, 10% glycerol, 1 mM DTT, 0.1 mM EDTA, made up to 2.5 mM $CaCl_2$ and digested with bovine thrombin at a mass ratio of 1:10 (thrombin: GST ORF104) for 2.5 hrs at 28°C to cleave the GST domain from the ORF104 domain. The digestion was stopped by the addition of 1 mM PMSF, 1 mM benzamidine and NaCl to a 1M final concentration. The digested protein was applied to a one ml glutathione sepharose column and flow-through fractions of 1 ml were

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collected. The fractions were analyzed by 12% SDS-PAGE (Tricine) and visualized by staining with Coomassie Brilliant Blue R250 stain to determine which fractions contain bacterially expressed ORF104 lacking the GST tag.

C. Affinity column preparation.

GST and GST/ORF104 fusion protein were dialyzed overnight against Affinity Chromatography Buffer (ACB; 20 mM Hepes pH 7.5, 10 % glycerol, 1 mM DTT, and 1 mM EDTA) containing 1 M NaCl. ORF104 protein obtained from thrombin digestion of GST/ORF104 was used without dialysis. Protein concentrations were determined by Bio-Rad Protein Assay and crosslinked to Affigel 10 resin (Bio-Rad) at protein/resin concentrations of 0, 0.1, 0.5, 1.0, and 2.0 mg/ml. The crosslinked resin was sequentially incubated in the presence of ethanolamine, and bovine serum albumin (BSA) prior to column packing and equilibration with ACB containing 75 mM NaCl.

D. *S. aureus* extract preparation.

Two extracts were prepared from *S. aureus* cell pellets. One lysate was prepared by French Pressure cell followed by sonication and the other by a lysostaphin digestion followed by sonication. The French Pressure cell prepared lysate was prepared by suspending 3g of frozen *S. aureus* cells in ACB containing 500 mM NaCl, 1 mM PMSF, and 1 mM benzamidine. The suspended cells were subjected to three passes through the French Pressure cell followed by 3 sonication bursts of 20 seconds each, made up to 0.1% Triton X-100, stirred for 30 minutes, and centrifuged at 50 000 rpm for 3 hrs in a Ti70 fixed angle Beckman rotor. The efficiency of cell lysis was low and the resulting lysate (7 ml) contained 2.4 mg/ml protein. The cell pellet after the French Pressure cell lysis was subjected to cryogenic grinding in liquid nitrogen in the same buffer with a mortar and pestle. The lysate was made up to 0.1% Triton X-100, stirred for 30

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minutes, and centrifuged at 50 000 rpm for 3 hrs in a Ti70 fixed angle Beckman rotor yielding a lysate (10 ml) containing 2.0 mg/ml protein. The cell lysates were found to be similar by SDS PAGE and were pooled, concentrated to 8 ml, and dialyzed overnight in a 3000 Mr dialysis membrane against affinity chromatography containing 1 mM PMSF, 1 mM benzamidine, and 75 mM NaCl. The dialyzed protein extract was removed from the dialysis tubing, centrifuged at 10 000 rpm in a Sorval SS34 rotor for 1 hr, and assayed for protein content (Bio-Rad Protein Assay) and salt concentration (conductivity meter).

The second lysate was prepared by lysostaphin digestion followed by sonication. A *S. aureus* cell pellet (2.9g) was suspended in 8 ml of 20 mM Hepes pH 7.5, 150 mM NaCl, 10% glycerol, 1 mM DTT, 1 mM PMSF, 1 mM benzamidine, and 1000 units of lysostaphin. The cell suspension was incubated at 37°C for 30 minutes, cooled to 4°C, and made up to a final concentration of 1 mM EDTA and 500 mM NaCl. The lysate was sonicated on ice using three bursts of 20 seconds each. The lysate was made up to 0.1% Triton X-100, stirred for 30 minutes, and centrifuged at 50 000 rpm for 3 hrs in a Ti70 fixed angle Beckman rotor. The supernatant was removed and dialyzed overnight in a 3000 Mr dialysis membrane against ACB containing 75 mM NaCl, 1mM benzamidine, and 1 mM PMSF. The dialyzed protein extract was removed from the dialysis tubing, centrifuged at 10 000 rpm in a Sorval SS34 rotor for 1 hr, and assayed for protein content (utilizing the Bio-Rad Protein Assay) and salt concentration (utilizing a conductivity meter). Aliquots of the extracts were frozen at -70°C.

E. Affinity chromatography.

S. aureus extract (400 µl) was applied to 40 µl columns containing 0, 0.1. 0.5. 1.0, and 2.0 mg/ml ligand and ACB containing 75 mM NaCl (400 µl) was applied to an additional column containing 2.0 mg/ml ligand. The columns were washed with ACB containing 75 mM

NaCl (400 µl) and sequentially eluted with ACB containing 1% Triton X-100 and 75 mM NaCl (160 µl), ACB containing 250 mM NaCl (160 µl), ACB containing 1M NaCl (160 µl), and 1% SDS (160 µl). 40 µl of each eluate was resolved by 16 cm 12.5% SDS-PAGE (Laemmli) and the eluted proteins were visualized by silver stain.

F. Identification of *S. aureus* DnaI homolog as an ORF104 interacting protein

Proteins at approximately 38 kDa were observed specifically in the eluants from the GST/ORF104 and ORF104 (GST removed) columns obtained from ACB containing 75 mM NaCl and 1% Triton X-100, and 1% SDS (Figs. 8-10; eluting protein indicated by an arrow). These bands were excised from the SDS-PAGE gels and prepared for tryptic peptide mass determination by MALDI-ToF mass spectrometry (Qin, J., *et al.* (1997) *Anal. Chem.* **69**, 3995-4001). High quality mass spectra were obtained (Fig. 11). The candidate proteins observed in the two eluants were identical as determined by the masses of the tryptic peptides (Fig. 11). Post-Source Decay (PSD) coupled with Collision-Induced Decay (CID) was used to obtain fragmentation spectra of tryptic peptides having monoisotopic MH+ masses of 1351.8, 1412.7, and 1617.8 Da. The fragment masses were used to search all public domain databases resulting in no identification. The PSD/CID spectra obtained for the peptide having a monoisotopic MH+ mass of 1412.7 were then interpreted to obtain a peptide sequence GHVPENVTDNDR (SEQ ID NO: 10), which was used to BLAST search the *S. aureus* nucleotide sequence database at http://www.genome.ou.edu/staph.html. One nucleotide sequence, Contig 981, in reading frame +3 encoded the similar amino acid sequence GHVPELYVDNNR (SEQ ID NO: 11). This tentative identification of the candidate protein was then confirmed upon conceptual translation and *in silico* tryptic digestion of the open reading frame found in Contig 981. Furthermore, the obtained PSD/CID spectra for tryptic peptides with monoisotopic MH+ masses of 1351.8 and

1617.8 Da were similar to the predicted PSD/CID fragmentation patterns of the tryptic peptides with monoisotopic MH+ masses of 1351.8 and 1617.8 Da found in the Contig 981 open reading frame. Comparison of the Contig 981 open reading frame with all other sequences in the public domain databases revealed that Contig 981 is a homologue of *Bacillus subtilis* DnaI, a protein involved in origin-dependent DNA replication (42% identity and 62% similarity) (Table 1).

G. Yeast two-hybrid confirmation of DnaI and ORF 104 interaction.

To validate the identification of the *S. aureus* dnaI homolog as an interacting partner of bacteriophage 77 ORF 104, the interaction was assessed *in vivo* in the yeast two-hybrid system. As shown in Fig. 12B, bacteriophage 77ORF104 was fused either to the carboxyl terminus of the yeast Gal4 DNA binding (pGBKT7, Clontech Laboratories) or activation (pGADT7, Clontech Laboratories) domains (pGBK77ORF104 and pGAD77ORF104, respectively). The polynucleotide sequence of the DnaI homologue was obtained from the *S. aureus* genomic DNA by PCR utilizing oligonucleotide primers that targeted the translation initiation and termination codons of the dnaI gene (SEQ ID NO: 1). As shown in Fig. 12A, the sense strand primer targets the initiation codon and is preceded by an *EcoRI* restriction site (5'-gaattc-3'); the antisense oligonucleotide targets the stop codon and is preceded by a *BamHI* restriction site (5'-ggatcc-3'). The PCR product was purified using the Qiagen PCR purification kit and digested with *EcoRI* and *BamHI*. The digested PCR product was ligated to *EcoRI*- and *BamHI*-digested pGADT7 vector (pGAD dnaI). A similar strategy was used for the cloning of DnaI into pGBKT7 vector (pGBK dnaI).

As shown in Fig. 12D, the pGAD and pGBK plasmids bearing different combination of constructs (as indicated in NO 1 to 6) were introduced into a yeast strain (AH109, Clontech Laboratories), previously engineered to contain chromosomally-integrated copies of *E. coli lacZ*

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and the selectable *HIS3* and *ADE2* genes. Co-transformants were plated in parallel on yeast synthetic medium (SD) supplemented with amino acid drop-out lacking tryptophan and leucine (TL minus) and on SD supplemented with amino acid drop-out lacking tryptophan, histidine, adenine and leucine (THAL minus). Co-transformants harboring the 77 ORF104 polypeptide only grew on selective THAL minus media in the presence of DnaI (right Petri, NO 5 and 6). Induction of the reporter *HIS3* and *ADE2* genes is dependent upon the interaction of dnaI with 77ORF104 proteins since when either plasmid is introduced into yeast host cells with the control plasmid (pGBKT7-53 orpGADT7-T), no reporter expression is observed (NO 2 and 3). pGADT7-T and pGBKT7-53 are positive control for protein:protein interaction (NO 1) and pCL1 is an active Gal4 transcription factor (NO 4). Interaction of DnaI and 77ORF104 is also demonstrated by the presence of luminescent β-galactosidase activity in 77ORF104-DnaI co-transformants (Fig.12E: NO 5 and 6). These results are consistent with the interpretation that the *S. aureus* DnaI homologue identified herein is the host target of bacteriophage 77ORF104.

Example 3

Identification of the surface of interaction on *S. aureus* DnaI.

To identify the specific domain of *S. aureus* DnaI which participates in the interaction with bacteriophage 77ORF104, recombinant DnaI protein was subjected to partial proteolytic digestion and applied to an affinity column containing 77ORF104. Partial proteolytic fragments of DnaI interacting with the 77ORF104 were then analyzed by SDS-PAGE and mass spectrometry, and subsequently characterized by yeast two-hybrid assay to validate the interaction of the DnaI sub-fragment with 77 ORF 104 as described in detail below.

A. Sub-Cloning of DnaI into a bacterial inducible expression system

Full-length DnaI, was amplified from *S. aureus* genomic DNA using the polymerase chain reaction (PCR). For PCR amplification of DnaI, the sense strand primer targets the initiation codon and is preceded by a *BamH*I restriction site (5'-ggatcc-3'); the antisense oligonucleotide targets the stop codon and is preceded by a *Sal*I restriction site (5'-gtcgac-3') (SEQ ID NO: 1). The digested PCR product was purified using the Qiagen PCR purification kit, ligated into *BamH*I and *Sal*I digested pGEX-6P-1 vector (# 27-4597, Amersham Pharmacia Biotech), and used to transform *E. coli* strain BL21. The sequence integrity of DnaI polypeptides fused to GST was verified directly by DNA sequencing.

Expression of the GST-DnaI recombinant protein from the plasmid pGEX-6P-1-DnaI was induced by the addition of 0.5 mM IPTG to a 6 liter culture at OD_{600} ~0.5. The protein was expressed at 30°C for 3h, the cells were harvested by centrifugation and stored as a cell pellet at –70°C. The frozen cell pellet was thawed, resuspended in Buffer 1 (20 mM HEPES pH 7.3, 500 mM NaCl, 10% glycerol, 1 mM DTT, and 1 mM EDTA) containing 1 mM PMSF and 1 mM benzamidine, and lysed in a French pressure cell followed by three sonication bursts of 20 seconds each at 4°C. The cell lysate was centrifuged at 4°C for 30 minutes at 10 000 rpm. The supernatant was applied to a 6 ml glutathione sepharose column equilibrated with Buffer 1, washed with 60 ml of Buffer 1 containing 1 mM PMSF and 1 mM benzamidine, and eluted in 6 ml fractions with Buffer 1 containing 50 mM reduced glutathione. Fractions were analyzed by 12% SDS-PAGE and visualized by Coommassie Brilliant Blue R-250 staining.

B. Cleavage and removal of GST fusion and partial proteolysis of DnaI

Elution fraction 5 containing 7.0 mg GST-DnaI was dialyzed against Buffer 2 (20 mM HEPES pH 7.5, 150 mM NaCl, 10% glycerol, and 1 mM DTT) and subjected to digestion with 40 Units precision protease (Amersham Pharmacia Biotech) at 25°C for 4 hrs. The digested

GST-DnaI was applied to a 1 ml glutathione sepharose column equilibrated with Buffer 2, the flow-through collected, and eluted with Buffer 1 containing 25 mM reduced glutathione. Fractions were analyzed by 12% SDS-PAGE and visualized by Coommassie Brilliant Blue R-250 staining.

The flow-through fraction, containing DnaI, was dialyzed against buffer 2 and subjected to proteolytic digestion in reactions containing the protease/DnaI mass ratio of 1:500 (w/w) of chymotrypsin or 1:50 (w/w) of endoproteinase Glu-C for 2h at room temperature. The partial proteolysis products obtained from chymotrypsin and endoproteinase Glu-C digestion were used for affinity chromatography. The proteolytic digestion was stopped by the addition of 1 mM PMSF and 1 mM benzamidine and analyzed by SDS-PAGE (one tenth of reaction used for analysis).

C. Affinity chromatography between immobilized 77 ORF 104 and DnaI proteolytic fragments

77ORF104 protein was cross-linked to Affigel 10 (BioRad) followed by blocking of the remaining active sites with ethanolamine and the non-specific sites with BSA. The columns were equilibrated with ACB containing 1 M NaCl, and ACB containing 100 mM NaCl. The partial proteolytic digests were diluted to a final volume of 120 µl with ACB containing 100 mM and purified BSA was added to a final concentration of 0.1 mg/ml. The partial proteolytic reaction was split into three fractions, of which 50 ul was applied to a column containing 77ORF104 crosslinked at 2.0 mg/ml, 50 ul was applied to a column containing no ligand, and 10 ul was retained for SDS-PAGE. The columns were washed with 10 column volumes of ACB containing 100 mM NaCl, 4 column volumes of ACB containing 100 mM NaCl and 1% Triton X-100, and eluted sequentially with 4 column volumes ACB containing 1 M NaCl and 1% SDS.

The flow-through and eluates were precipitated with trichloroacetic acid (TCA) and washed with an equal volume of cold (-70 °C) acetone. The TCA-precipitated samples were subjected to 15% SDS-PAGE, and the protein visualized by silver staining (Fig. 14 A and B).

D. Identification of DnaI partial proteolytic fragments interacting with 77 ORF 104

The interacting proteolytic fragments were excised, digested by trypsin, and analyzed by mass spectrometry. The peptides contained within each of the interacting proteolytic fragments were analyzed by MALDI-ToF mass spectrometry resulting in the determination of the general region of DnaI for each partial proteolytic peptide. The amino and carboxy terminal ends of the partial proteolytic fragments were determined for several fragments by the acquisition of mass spectrometry data of the unfractionated proteolytic digest followed by mapping the observed mass onto the full length DnaI sequence. Partial proteolytic DnaI fragments interacting with the 77 ORF 104 are presented in Fig. 14C.

E. Sub-Cloning of DnaI fragments into yeast inducible expression system

The interaction between 77ORF104 and portions of the DnaI polypeptide was also assessed *in vivo* in the yeast two-hybrid system. Two portions of the polynucleotide sequence of DnaI were amplified by PCR from *S. aureus* genomic DNA by utilizing appropriate pairs of oligonucleotides (Fig. 15). The portion extending from amino acid residues 64 to 313 was obtained with the two following oligonucleotides: the sense strand (with an *Eco*RI cloning site) 5'- ccggaattc TATAAAGATCAACAAAAAC-3', SEQ ID NO: 12 and the antisense strand (with a *Bam*HI cloning site) 5'- cgcggatccTCAATTGTTTCTGAAATT- 3', SEQ ID NO: 13.

The polynucleotide sequence encoding amino acids 150-313 of SEQ ID NO: 2 corresponds to nucleotides 448 to 942 of SEQ ID NO: 1 and is herein designated SEQ ID NO: 17. The portion extending from amino acid residues 150 to 313 was obtained with the two following

oligonucleotides: the sense strand (with an *Eco*RI cloning site) 5'-ccggaattcGCAGCAGATGATATTTGT -3', SEQ ID NO: 14and the antisense strand (with a *Bam*HI cloning site) 5'- cgcggatccTCAATTGTTTCTGAAATT -3', SEQ ID NO: 15. The digested PCR products were gel purified, ligated into *Eco*RI- and *Bam*HI-digested pGADT7 prey vector, and used to transform *E. coli* strain DH10β. The sequence integrity of the cloned products was verified directly by DNA sequencing.

As shown in Fig. 16 different combinaison of bait and prey vectors (as indicated in NO 1 to 6) was introduced into AH109 yeast cells. Portions of DnaI extending from amino acids residues 64 to 313 (herein referred to as SEQ ID NO: 18) as well as from 150 to 313 (herein referred to as SEQ ID NO: 16) were both found to interact with bacteriophage 77ORF104 since the introduction of appropriate plasmids into host yeast cells resulted in their growth on THAL minus SD medium (NO 1 and 3). Induction of these reporter genes is dependent upon the interaction between DnaI-related polypeptides and 77 ORF 104 since the introduction of control plasmids expressing non-interacting protein partners (pGBKLam: NO 2 and 4 or 77pGADORF13: NO 6) did not result in reporter gene expression (Fig. 16A).

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OTHER EMBODIMENTS

Other embodiments are within the following claims.